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BIOLOGICAL BULLETIN

RESPIRATORY DIFFERENCES ALONG THE AXIS OF THE SPONGE *GRANTIA*.

LIBBIE H. HYMAN

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Differences in the rate of respiratory metabolism along the principal axis have now been demonstrated for a number of animals: for the hydroid Corymorpha (Child, '23, Hyman, '23*a*), for the medusa Cassiopaea (McClendon, '17), for Planaria (Hyman, '23*b*), and for several annelids (Hyman and Galigher, '21). Recently Shearer ('24) has reported similar results for the chick embryo and the earthworm. Unfortunately in Shearer's work regions of very different morphological constitution were compared and it is therefore doubtful if his results can be regarded as lending support to the physiological gradient conception. For example anterior and posterior halves of the chick embryo in the stages studied by Shearer differ enormously in their content of nervous tissue, due to the presence of the brain and chief sense organs in the anterior half. The much greater respiratory rate of the anterior half reported by Shearer is probably largely due to the greater proportion of nervous tissue which it contains. Similarly in the earthworm the mature head is morphologically and functionally different from the rest of the body and respiratory differences between it and other regions must be in part due to such specific differences. In brief, Shearer's measurements concern not the gradient itself but the secondary differentiations associated with the gradient. Shearer also seems to be unaware of the existence in annelids (and in the early embryos of vertebrates) of the double type of gradient (Hyman and Galigher, '17) and the pieces of the earthworm which he compared were consequently not correctly chosen and serve neither to prove nor disprove the existence in this animal

of the type of gradient which we have described for it. The acetone powder experiments do not, in my opinion, answer these objections. If the substance in such powders which absorbs oxygen is essential to respiration then it is necessarily true that types of tissues which have a high respiratory rate must contain a higher percentage of the substance. It has been repeatedly emphasized that direct proof of axial respiratory differences can be obtained only through the comparison of pieces of like morphological constitution and through the elimination of various functional factors which affect metabolic rate. It is to be hoped that Shearer will repeat these experiments with more suitable material. Such material is necessarily limited to the lower invertebrates or to the very early embryonic stages of higher forms.

The sponge *Grantia* seemed to me to constitute very favorable material for a further test of the reality of axial metabolic differences. It has the same morphological constitution throughout except at osculum and base and is more or less definitely polarized. The work was performed at the Marine Biological Laboratory at Woods Hole during the summer of 1924.

1. Method of Determining the Oxygen Consumption.—For two or three years I have been trying to devise an apparatus suitable for the study of the oxygen consumption of small organisms by Winkler's method. The method finally adopted owes its origin to a device described by Osterhaut and Haas ('17). They first suggested an apparatus separable into two pieces, one part to contain the organisms and the other part for analysis. Their apparatus is, however, clumsy to manipulate and for large animals or large amounts of material the method used by me for many years of siphoning off the sample is very much simpler and entirely satisfactory, as proved by checks. The device proposed by Osterhaut and Haas to prevent exposure to air in adding the reagents is in my opinion wholly unnecessary unless one is dealing with water of very low oxygen content. In working with small organisms, a very much smaller apparatus is required. This naturally reduces the size of the sample of water available for analysis. This difficulty is overcome by using a smaller quantity of the reagents and a more dilute thiosulphate solution for the final titration, as also suggested by Lund ('22).

The apparatus which I have been using has the following simple construction, illustrated in the accompanying figure. It consists of a Pyrex test tube *A*, without rim, 100 mm. long by 10–12 mm. diameter, capacity about 10 cc. This is surmounted by a piece of rubber tubing *B*, into which fits a short length (about 40 mm.) of Pyrex tubing *C* of the same diameter as the tube *A*. Over the end of *C* is another piece of rubber tubing *D*. The rubber tubing must fit tightly over the glass and if necessary *B* can be wired to *A* and *D* to *C*, but not *C* to *B*. A number of such outfits should be prepared. For the water blanks only the parts *A* and *B* are necessary.

FIG. 1.

The apparatus is used as follows. In those tubes which are to contain the animals all four pieces must be fitted together as in the figure and *C* must be shoved close to but not in contact with *A*. All of the tubes to be used in one experiment, including the tubes for the blanks, are filled by siphon with the same water from an elevated receptacle. The siphon should reach to the bottom of the tube and the water be allowed to flow out of the top for some time. The animals to be used can be placed in the tubes before they are filled with water if of suitable nature, or if not, can be put in after the filling. All tubes are then closed by screw clamps around *D* in the experimental tubes, or *B* in the blanks. The tubes are then placed in a suitable water bath kept at constant temperature. Those containing the animals should be agitated at intervals, in the case of non-motile animals, to prevent an accumulation of metabolic products and exhaustion of the oxygen around them. When it is desired to conclude the experiment, each experimental tube is inverted several times to insure uniform distribution of its oxygen content and is then finally inverted, the animals being brought by gravity into the section *C* plus *D*. A screw clamp of suitable size is then rapidly placed around the tubing *B* over the small interval left between *A* and *C*. On screwing the clamp, *A* and *C* will be found to move apart readily and the walls of the tubing *B* are at the same time drawn together by the negative pressure thus

developed. One should never try to pull the sections *A* and *C* forcibly apart. After screwing the clamp tightly, the sections *C* plus *D* are withdrawn from *B*, the apparatus being held inverted during the whole of the procedure to prevent the spilling of the contents of *C* plus *D*. The contents are immediately poured into a small graduated tube and the volume noted. Graduated centrifuge tubes have been found very convenient for this purpose. A little practice with the apparatus is required to prevent the spilling of the water from *C* plus *D* and a tube should be at hand to receive this water just as *C* is withdrawn from *B*. The portion *A* plus *B* is now analyzed by Winkler's method and the blanks which consist of only *A* plus *B* are analyzed at the same time. The clamp around *B* in each tube is open and 0.1 cc. of each of the reagents used in Winkler's method is added. The clamp is then closed, the contents shaken, the precipitate allowed to settle as usual, and after again opening the clamp, 0.1 to 0.2 cc. concentrated HCl added carefully so as not to disturb the precipitate. The clamp being again closed, the tube is shaken to dissolve the precipitate and the contents are then poured into a small evaporating dish and titrated with sodium thiosulphate. The latter should be about 1/500 normal. With greater dilution of the thiosulphate the end point becomes uncertain. The volume of the tube *A* plus *B* must then be determined. If care is taken not to move the clamp around *B* during the preceding operations, the tube *A* plus *B* can be filled with water of the same temperature as that obtaining during the experiment, the clamp closed, excess water at the top removed, and the contents then poured into a graduated tube. In my experience the volume of *A* plus *B* and *C* plus *D* must be determined at each experiment as they vary slightly owing to variations in the position of the clamp when the two portions are separated. One must remember to subtract 0.2 cc. from the volume of *A* plus *B*, to compensate for the loss of this amount of water when the reagents are added.

In calculating the results, it must be borne in mind that only the portions *A* plus *B* are analyzed while the animal respired from *A* plus *B* plus *C* plus *D*. The thiosulphate equivalent of each cc. of *A* plus *B* is calculated and from this the thiosulphate equivalent of the entire apparatus is obtained. It is thus neces-

sary to know the exact volume of *A* plus *B* and *C* plus *D*. The oxygen content at the beginning is determined in the same way by calculation from the water blanks and is brought by ratio to the same volume as the experimental tube. A simple subtraction then gives the oxygen consumed by the animals in terms of thiosulphate. To find the oxygen equivalent of the thiosulphate the latter must of course be standardized. A number of methods are given in any text on quantitative analysis. If the oxygen consumption in terms of volume is desired, as is usually the case, then it is desirable to conduct all of the experiments at the same temperature and to determine the volume of the tubes for this temperature. The oxygen equivalent of the thiosulphate for any given temperature can be determined from data on the volume of oxygen at different temperatures given in handbooks of physical constants.

The apparatus can be modified in various ways to suit the type of animal employed. For Protozoa the tube is closed, after filling with water and adding the animals, not by means of a clamp but by means of a glass plug. This is inserted deeply into *D* and must be firmly wired in. At the end of the experiment the tube is placed in the centrifuge in the inverted position, the bottom of *A* pointing towards the axis of the centrifuge. By centrifuging, the Protozoa are driven into the section *C* plus *D*, which is then removed as already described. For larger animals the size of the apparatus can be increased or in place of the test tube *A* a small flask of desired content can be substituted. In such cases, the amount of the reagents used and the dilution of the thiosulphate should be adjusted to the size of the sample obtained for analysis. The apparatus is not suitable for animals which cling firmly, such as planarians.

The method is naturally not as accurate as the regular Winkler method. By the analysis of duplicate samples I have found that the oxygen content of 15 cc. of water—about 0.07 to 0.08 cc. at air saturation and room temperature—can be determined with an error of about 0.002 to 0.003 cc. This makes an error of some 3 to 4 per. cent. while in the regular Winkler procedure, with samples of at least 100 cc., the error is less than 0.5 per cent. The method is thus chiefly of value for comparative work.

2. Method of Determination of Carbon Dioxide Production.—For this purpose the simple method first suggested by Haas ('16) was employed. A set of standard tubes containing phenol red solutions covering the range of hydrogen ion concentration from pH 6.8 to 8.4 must be at hand. These are now sold by dealers in chemical supplies. For the experiments tubes of the same dimensions are necessary. The animals to be tested are placed in such tubes and those to be compared must be of the same weight. Phenol red in powder form is added to sea-water until the density of color is the same as that in the tubes of the standard set. An equal volume of this water is then added to each of the tubes containing the animals and the tubes then sealed with paraffin. If the inside of the tube above the water is wiped dry, paraffin will adhere to it firmly and the melted paraffin can be then added directly onto the surface of the water. The sealed tubes are placed in a water bath at constant temperature and the changes in tint due to the production of carbon dioxide by the organisms are recorded in terms of pH by comparison with the standard tubes. There is of course some error (probably about .2 pH) in working with sea-water unless the standard sets have been especially prepared for such work. This error is of no consequence in comparative work.

3. Material and General Procedure.—Only freshly collected sponges were employed and these were used as soon as brought in by the collectors. Only the cleanest and most perfect specimens were used; those selected were placed in a dish of clean sea-water and repeatedly squirted about with a pipette to free them as far as possible from foreign materials clinging to their surfaces. Unfortunately in the case of sponges it is not possible to determine by inspection whether the specimens are in good physiological condition or not. The selected specimens were placed on a glass plate, osculum and base cut off and discarded, and the body then cut into two nearly equal halves. These pieces were then placed in the tubes for the respiration tests.

It was of course necessary to weigh the pieces. They were gently rolled about on hard filter paper until they no longer wet the paper, then transferred to small weighing bottles, previously weighed, and weighed to the fourth place. In the case of the oxygen consumption tests, the pieces were weighed after the conclusion of the experiments. For the carbon dioxide pro-

duction tests, it is necessary to weigh the pieces in advance since by this method only pieces of equal weight can be compared. The sponges were cut into slightly unequal portions, and the smaller portion weighed first. The larger portion was then weighed and small pieces removed from it until its weight equalled that of the other piece. After such handling it was thought advisable to allow the pieces to stand in water for two or three hours before beginning the tests and this was always done.

The sea-water used in all of the experiments was kindly furnished to me by Dr. A. J. Goldfarb. This water was collected from the end of the Bureau of Fisheries pier at Woods Hole and stood for several days prior to its utilization to allow debris to settle. This water contained no organisms visible to the eye but no doubt some bacteria were present. As the blanks however are allowed to stand as long as the experimental tubes before analysis, this possible source of oxygen loss is cancelled out. The water was thoroughly aërated before use and was thus saturated with air at the beginning of every experiment.

In nearly all experiments two pieces of sponge were placed in each tube, such pieces being of course from the same level of the sponges concerned. Thus for each experiment two sponges were selected and cut and the two apical halves placed in one tube, the two basal halves in the other. The tubes were then filled by siphon as already described. In some cases they were filled first and the pieces of sponges dropped in afterwards. In the experiments on carbon dioxide production, the pieces were placed in the tubes and with a pipette a definite amount of sea-water colored with phenol red run into each tube. At first five cc. of water were added to each tube but the carbon dioxide production was found to be so slow that later only two cc. were employed.

The control of temperature was difficult at Woods Hole. Owing to the lack of constant temperature apparatus, the experiments had to be run at room temperature. At the beginning of each oxygen consumption experiment, the water and water bath were allowed to come to room temperature and thereafter the bath was kept at this temperature by adding warm or cold water as the case might be. The carbon dioxide experiments,

however, ran over such long periods of time in many cases, that the temperature could not be kept constant by personal attention and varied during the night with changes in the air temperature.

4. Results.—The results of the oxygen consumption experiments are given in Table I. Thirteen experiments were per-

TABLE I.

OXYGEN CONSUMPTION OF APICAL AND BASAL HALVES OF THE BODY OF THE SPONGE *Grantia*.

Duration of each experiment three hours. Temperature in different experiments 21 to 23° C. Oxygen given in cubic centimeters.

No. of Exp.	Kind of Piece.	O_2 Content at Start.	O_2 Content at End.	Oxygen Consumed.	Weight.	O_2 Consumed per Gram per Hr.
1	Apical	0.080	0.072	0.008	0.0193	0.138
	Basal	0.082	0.071	0.011	0.0265	0.138
2	Apical	0.077	0.061	0.016	0.0345	0.154
	Basal	0.074	0.057	0.017	0.0360	0.157
3	Apical	0.077	0.063	0.014	0.0285	0.163
	Basal	0.069	0.059	0.010	0.0319	0.104
4	Apical	0.074	0.053	0.021	0.0578	0.121
	Basal	0.074	0.049	0.025	0.0730	0.114
5	Apical	0.082	0.070	0.012	0.0682	0.058
	Basal	0.077	0.067	0.010	0.0732	0.043
6	Apical	0.080	0.069	0.011	0.0452	0.081
	Basal	0.074	0.062	0.012	0.0536	0.074
7	Apical	0.077	0.069	0.008	0.0310	0.086
	Basal	0.077	0.069	0.008	0.0482	0.056
8	Apical	0.074	0.052	0.022	0.0442	0.166
	Basal	0.075	0.055	0.020	0.0452	0.147
9	Apical	0.074	0.055	0.019	0.0388	0.163
	Basal	0.071	0.060	0.011	0.0428	0.086
11	Apical	0.074	0.053	0.021	0.0510	0.137
	Basal	0.075	0.055	0.020	0.0581	0.114
12	Apical	0.073	0.057	0.016	0.0320	0.166
	Basal	0.066	0.053	0.013	0.0406	0.106
13	Apical	0.072	0.054	0.018	0.0392	0.153
	Basal	0.073	0.051	0.022	0.0558	0.131

formed, of which one (No. 10) was lost. The table shows the oxygen content of the whole tube ($A + B + C + D$) at the

beginning and at the end of each experiment. The former is determined by calculation from the control blanks, and the latter by calculation from the analysis of the portions *A* plus *B* of the experimental tubes, the contents of *A* plus *B* and *C* plus *D* being known. When calculated in this form, the difference

TABLE II.

RELATIVE CARBON DIOXIDE PRODUCTION OF APICAL AND BASAL PIECES OF THE BODY OF THE SPONGE *Grantia*, IN TERMS OF TIME REQUIRED TO CHANGE PHENOL RED FROM pH 8.0 TO 7.5.

h., hours; m., minutes.

No. of Exp.	Kind of Piece.	Weight, Grams.	Time for Change from 8.0 to 7.5.	Remarks.
1	Apical	0.051	5 h., 50 m.	5 cc. sea-water used.
	Basal	0.058	9 h., 40 m.	
2	Apical	0.0212	21 h., 30 m.	5 cc. End point of basal piece not exactly determined.
	Basal	0.0234	25 h., plus	
3	Apical	0.0314	20 h., 40 m.	5 cc.
	Basal	0.0318	24 h., 55 m.	
4	Apical	0.0217	21 h.	4 cc. Repeated with same result.
	Basal	0.0214	16 h.	
5	Apical	0.019	26 h., 15 m.	5 cc. Almost no difference between them at any time.
	Basal	0.019	26 h., 15 m.	
6	Apical	0.0182	15 h., 40 m.	4 cc.
	Basal	0.0184	17 h., 30 m.	
7	Apical	0.0179	5 h., 15 m.	2 cc.
	Basal	0.0174	8 h.	
8	Apical	0.0212	6 h., 25 m.	2 cc.
	Basal	0.0226	8 h.	
9	Apical	0.0313	5 h., 30 m.	2 cc. Basal piece in advance on next day.
	Basal	0.0300	9 h.	
10	Apical	0.0218	8 h., 10 m.	2 cc. Even next day.
	Basal	0.0234	9 h., 40 m.	
11	Apical	0.0112	17 h.	2 cc. No difference between them until after 4 hrs.
	Basal	0.0124	19 h.	

between the two values gives the oxygen consumed by the pieces. This divided by the weight and then by three (as each experiment ran for three hours) gives the oxygen consumed per gram of weight per hour. The figure thus obtained seems a little low as compared with the respiration rate of other animals

—it is about half of that of Planaria—but it must be remembered that a considerable portion of the weight of the sponge is due to the lifeless spicules.

Of the twelve experiments presented in Table I., the rate of oxygen consumption is markedly greater in the apical piece in five cases (Nos. 3, 5, 7, 9, and 12), slightly greater in five cases (Nos. 4, 6, 8, 11, and 13) and about equal to that of the basal piece in two cases (Nos. 1 and 2). In experiments 4 and 6 the difference between the apical and basal pieces is so slight as probably to be of no significance.

The experiments on carbon dioxide production yield about the same result. In nine cases, the apical pieces produce carbon dioxide at a faster rate than do the basal pieces, in one case (No. 5) the rate is equal and in one case (No. 4) the result is the reverse. The advantage in favor of the apical piece is not very great in Nos. 10 and 11.

These findings are in agreement with the electrical differences previously discovered in this sponge (Hyman and Bellamy, '22). It was found that in the majority of individuals the oscular end is electropositive (internally) to more proximal regions, but that in some individuals this potential difference is absent or may even be reversed. That these electrical differences are correlated with the metabolic differences is a view which I have held for a number of years. It is presumable that those individuals in which electrical or metabolic gradients are lacking are in poor physiological condition.

Finally attention may be called to the relation between rate of oxygen consumption and size (weight). In general, the greater the weight of the pieces, the lower is the rate of oxygen consumption. This inverse relation between size and respiratory rate seems to be general throughout the animal kingdom (cf. Hyman, '19).

5. *Summary.*—In the majority of cases, apical pieces of the body of the sponge *Grantia* consume oxygen and produce carbon dioxide at a higher rate per unit weight than do basal pieces. This result furnishes further evidence in favor of the axial gradient conception.

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THE DISTRIBUTION OF CERTAIN INSECTS OF REVERSED BEHAVIOR.*

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The recent researches of Loeb, Holmes and other students of animal behavior as well as the work of various entomologists on the reactions of insects to definite environmental factors, has given us a mechanistic interpretation of insect activities very different from the anthropomorphic interpretation of the earlier students. Shelford, Dean and others have related many insect adjustments in behavior with remarkable exactness to specific conditions of light, temperature, humidity, etc.

These reactions are frequently quite specific but many of them are the same for all the species of a genus. As, for instance, all species in a genus are nocturnal or all are aquatic. Such a generic tropism usually defines the generic habitat in a broad way. Within this general habitat the individual species will have individual habitats limited by other tropisms. Apparently the generic tropism that defines the generic habitat is seldom modified for any individual species enough that such a species may exist outside the generic habitat. But apparently a complete reversal of a generic tropism is more likely of occurrence than any lesser modification. When such occurs in a genus the individual species possessing this reversed generic tropism has entrance into an environment closed to all other members of its genus.

The writer has come across two species of insects, one a dragonfly, the other a mayfly, in which a reversal of one or more of the tropisms normal to the other species of the same genus has permitted the entrance of these reversed species into environments not open to the normal members of the genus. These finds have opened up so many interesting problems in behavior and distribution that they are well worth presenting in some detail.

*Contribution from the Department of Zoölogy and Entomology, Ohio State University, number 79.

The first of these that was found is *Æshna nevadensis* Walker of the Sierra upland of central California.[1] This is a large, pond and shallow lake dragonfly that lives at elevations of 5,000 to 9,000 feet. It is known only from the southern Sierra Mountains. It has been recorded from Walker Lake, Mono Co., California, at 7,700 feet elevation, Hardin Lake, Tuolumne Co., at 7,775 feet elevation and from Elizabeth Lake, Yosemite National Park at the great altitude of 9,000 feet. The writer found it at its optimum development in shallow, weed-filled lakes on the divide between Lake Tahoe and the Rubicon River (Calif.) where at an elevation of 7,000 feet it dominated the subalpine dragonfly life. Here in an open, meadow-like mountain pass, fairly level for about two miles, lie four shallow lakes, two flowing into Lake Tahoe and the desert drainage, while the other two empty into the Rubicon River and the Pacific drainage. On both sides rise granite crags for a thousand feet above the lakes, their lower parts green with firs, while their gray upper slopes are blotched white with fields of snow. Three of the lakes are covered with yellow flowered pond lilies and are fringed with green sedges among clumps of silver gray willows. These shallow lakes are warm because of the black peat bottom and long hours of sunshine. They support great numbers of a few species of insects—such as are able to withstand the long winters and cold nights at this elevation, as this is a subalpine situation with nightly temperatures at or near freezing. Eight other species of dragonflies are common here. These are northern forms that occur in the mountains of Oregon at 3,000–4,000 feet elevation and in British Columbia at sea level.

The habitat of *nevadensis* appears to be entirely above that of all other species of North American Æshnas. It appears as strayed individuals at 4,500 feet but does not appear in numbers until an elevation of 6,000–7,000 feet is reached. From here up to 9,000 feet it appears at its optimum development. No other Æshnas have been found regularly at these higher altitudes. From about 4,500 feet down to sea level, however, Æshnas are a constant element of the North American Odonate fauna. Twenty or more lowland species have been described from north of Mexico while there are actually seven or eight species living

[1] Kennedy, *Proc. U. S. Nat. Mus.*, Vol. 52, pp. 483–635.

about the lower slopes of the Sierras. Thus *nevadensis* has a habitat entirely above or outside of that of the other North American members of the genus.

We do not know the behavior complex of *Æshna nevadensis* in enough detail to speculate on all the adjustments in reactions necessary to adapt this species of a lowland genus to a highland habitat. It shares with all other Æshninæ, and without any change, a positive reaction to shining water surfaces when the sexual instinct is not overbalanced by hunger. This is a reaction through the eye as *Anax*, a related genus, was found reacting just as positively to the glistening surfaces of the crude oil pools of the Bakersfield (Calif.) oil field where hundreds of *Anax junius* perished while mating and trying to oviposit in the crude oil. When hunger predominates over the sexual impulse the reactions change so that the Æshnas fly away from the water on hunting trips into the surrounding territory. They fly away from the water and roost on trees when the minimum flying temperature is reached, also at twilight on warm evenings when the minimum flying light is reached. The minimum flying light varies greatly with the different species as some will still fly when it is so dark to the human eye that the dragonfly can be seen only when it is outlined against the sky or some white surface.

As ponds for oviposition are the same on the Sierra upland as at sea level, we find the reactions of *nevadensis* while under the sexual impulse practically identical with the similar reactions of the lowland species. The adjustments to the upland come in the reactions of the insects when hunger and other impulses outweigh the sexual impulses, and have nothing to do with the coursing of the males and females over the surface of the water while mating and ovipositing.

Two of these adjustments to the conditions found in these high altitudes are quite obvious. First the hunting individuals react negatively to the warm stratum of air next the ground so that, except early in the morning when the ground stratum of air is still cool, they hunt high off of the ground flying from fifteen to one hundred and fifty feet in the air. It is a tree-top species. This positive reaction to cool air probably explains the attraction of *nevadensis* to this alpine habitat. All other species of Æshna,

when hunting, react negatively to the cooler situations of the habitat, the warmer the place the better, until 100–105° is reached when some species begin to become inactive.

The second obvious adjustment necessary is in the time of emergence. All lowland species of Æshna, as far as known, emerge at night, those the writer has reared, *umbrosa* and *constricta*, emerging at or near midnight. This appears to be an adjustment of value in that the imagoes have hardened sufficiently by daylight for flight and so escape the blackbirds and other marsh fowl that enjoy soft freshly emerged insects. But at an elevation of 7,000–9,000 feet with snow fields spread about, the nightly temperatures are never far above freezing and frequently below, making night emergence precarious as the young dragonfly would be too chilled to crawl out of its nymphal skin. Here a second adjustment to this elevated habitat appears. The emerging nymph of *nevadensis* instead of being negatively geotropic in the dark is negatively geotropic in the light, so that the winged adult emerges in the broad daylight when the temperature is high enough to insure a successful withdrawal from the nymphal skin. The writer found numerous individuals emerging in the bright sunshine in the early afternoon hours.

Thus we see that *Æshna nevadensis* has left the general warm lowland environment of the genus and has entered an entirely different region through having two of the tropisms normal to Æshna reversed.

While in the mountains of eastern Tennessee last spring, the writer discovered a mayfly, *Ephemera guttulata* Pictet, which appears to occupy a habitat entirely different from that of any other species of Ephemera. It too appears to have certain of its reactions the reverse of those of the other species of this genus.

Ephemera guttulata is a most interesting mayfly in several ways. It is one of our large mayflies. Its wings are so heavily clouded that at a little distance they appear almost black, especially as contrasted against the abdomen, which is immaculate snow-white. This bizarre insect lives in the smaller of the perennial, spring-fed mountain torrents that flow down the higher of the Eastern Tennessee Mountains. On Chilhowee Mountain these streams pour down deep V-shaped ravines over beds of small stones and coarse grit, in a succession of miniature waterfalls, for they

descend at a rate of several hundred feet to the mile. These mountains are covered with pines on their high, dry ridges but the deep ravines between these ribs of pine woods are filled with a dense growth of deciduous timber so that the torrents are heavily shaded by tall trees in their whole course.

The burrowing larva of *guttulata* lives in the meager areas of coarse sand and muck found in the little basins below the waterfalls. The subimago emerges during the day. Those the writer observed came out on dull cloudy days. These fly out of the shade over the stream, through the surrounding brush and up to the better lighted areas of the hill side where they rest in the full light. The dull gray subimago then sheds a thin skin and comes out a fully developed imago with its brilliant black and white colors and its sexual maturity. No observations were made as to whether this occurred on the day of emergence or the following day. Because of the few subimagoes seen it probably occurred the same day as the emergence. Unfortunately also, no mating dances were seen. These probably occurred among the tree tops, in the deep dusk, just before egg laying began.

When the evening twilight had deepened to the point where it became difficult to pick one's way along the streams, the females of *guttulata* would appear over the little pools hurrying back and forth about a foot above the surface of the water apparently laying eggs. Their conduct was more like that of female dragonflies than like the usual hurried visit of the mayfly female dropping all of her eggs in a single effort. No males were caught at these times over the streams.

It was in these flights in the dense twilight gloom of the bottoms of these mountain gorges that the probable value of the bizarre coloration came to mind. The enormous development of the eyes, the evidence of the rudimentary antennæ together with certain experimental work indicate that the major reactions of the mayflies are through the sense of sight. Except for the white abdomen, the mayflies, at the time of these twilight flights, were practically invisible to the observer. These white abdomens, as the *guttulata* females doged about in the gathering darkness, reminded one of the streaks of light of a flight of fireflies. Apparently then this white abdomen is useful to *guttulata*

in the mating flights in the deep shade of the mountain gorges as its visibility is very obviously increased by this color pattern. A snuff-colored, lowland Ephemera would be practically invisible under the same conditions. It is interesting to note that of several species of mayflies flying on these streams at this time *guttulata* is the latest on the wing in the evening and flies after the others have ceased. Some of the smaller species fly a full hour earlier, so that, though, of dull colors they are quite visible.

We can check this series of reactions of *guttulata* by a comparison with the reactions of the other species of Ephemera, all of which inhabit either large open streams or lakes. Probably the reactions of *Ephemera simulans*, the common Lake Erie species, are best known. The nymph of this species burrows in the mud of the lake bottom, being obviously negatively phototropic. At the time of emergence it becomes positively phototropic and rises to the light of the sky. At Put-in-Bay this emergence takes place between eight and ten P.M. It sheds its skin as it rises through the ten to thirty feet of water so that on arrival at the surface it bursts out fully winged, when it becomes less positively phototropic and flies toward the dark land. It rests on the shore vegetation until the following evening when it sheds its final skin, becomes sexually mature and at twilight flutters up and down in the mating dance. At this stage it is evidently becoming positively phototropic again. In this twilight nuptial dance it leaves the dark foliage for the more open lighter spaces. The males grasp the females and release them after a contact of a few seconds. The female becomes at once completely, positively phototropic and flies out toward the light surface of the lake to deposit her eggs.

If we compare this series of reactions with those of *guttulata* of the shaded mountain streams, we find that two of the series of reactions of the latter are reversed. *Guttulata* is negatively phototropic as a nymph, is positive as it emerges, but ***remains positively phototropic after emergence*** as it flies from the heavily shaded creek to the lighter areas above the shade. Further, ***after copulation it becomes negatively phototropic*** and flies down to the densely shaded torrent to oviposit. Any of the open stream species of Ephemera would react themselves away from the

shaded stream when they started to oviposit. So by these reversed reactions *guttulata* is able to occupy a habitat that the normal members of the genus *Ephemera* are not able to occupy, one that is ecologically outside of the general habitat of the genus.

Certain experimental studies in the behavior of mayfly nymphs point to possible explanations of some of this series of reactions. Wodsedalek [2] has demonstrated that the nymph of Heptagenia is negatively phototropic. Also that CO_2 in the water will make a nymph which has been negatively phototropic, reverse its reaction and become positively phototropic. It is then possible that the change in reaction at the time of emergence, when the nymph ceases to burrow and swims up to the light, is due to an accumulation of CO_2 in the nymph after its tracheal system has detached itself from the gills in the last nymphal ecdysis. The shedding of the skin actually starts by a general loosening of the chitin several hours before the final emergence occurs. During this time, if the gills are early detached, much CO_2 could accumulate in the tissues, enough eventually to reverse the phototropism and so cause the nymph to rise to the surface.

On emergence the nymph fills its tracheæ with air and simultaneously becomes negatively phototropic, so that it flies toward dark land. It appears to retain this reaction until the next evening at dusk or for about twenty hours when it mates and the female at once becomes positively phototropic and flies towards the light surface of the open lake. During the intense nuptial dance her tissues have been accumulating CO_2 and in some way the sexual orgasm overbalances the condition, giving the acid condition full sway.

The experiments of Allee and Stein [3] show that the reactions are not as simply explained as they have been sketched in the preceding paragraphs. The actual intensity of the light probably also figures in some of the reactions. Krecker's work [4] in the subimagoes of *Hexagenia*, a close relative, shows that these, in spite of flying to the dark land, are positive to certain bright

[2] Wodsedalek, "Phototactic Reactions and their Reversal in the Mayfly Nymphs of *Heptagenia interpunctata* (Say)," BIOL. BULL., Vol. 21, pp. 265–271, 1911.

[3] Allee and Stein, "Light Reactions and Metabolism in Mayfly Nymphs," *Jour. Exp. Zoöl.*, Vol. 26, pp. 423–458, 1918.

[4] Krecker, "Phenomena of Orientation Exhibited by Ephemeridæ," BIOL. BULL., Vol. 29, pp. 381–388, 1915.

lamps. Anyone who has seen the snow storms of mayflies that come to the street lamps of the lake ports realizes that the subimago can have a reverse tropism under such conditions.

We are beginning to recognize physiological species among insects—those based on habits and habitats. In parasitic insects particularly we recognize generic reactions to common hosts so that we unofficially recognize physiological genera. There is no reason why we should not, except the expediency of morphological characters. Viewed in this light *Æshna nevadensis* and *Ephemera guttulata* are physiologically outside of their respective genera.

When the writer first thought through the habits and distribution of the North American species of the genus *Æshna*, his conclusion was that the positive thermotropism of the lowland species was perhaps different in degree and distinct for each species, thus explaining the restriction of each species to its specific thermal belt. Thus it appeared, at first, that this difference in positive thermotropism accounted for the fact that some Æshnas lived in hot Arizona, others in the cooler northern states, while still others are restricted to the northern parts of Canada and Alaska. However on investigating further this does not appear to be true.

The restriction of each species to its specific thermal belt is probably not due to a limitation of the positive thermotropism of the adult to the narrow limits of the particular thermal belt inhabited by that species. This is quite contrary to Merriam's theory of distribution by thermal zones.[5]

This distribution of the various species in different thermal zones however is a very striking thing and some of the thermal conditions are easily sketched. Except for *mutata*, *californica* and *multicolor*, which are early spring species, the majority of the species of Æshna are on the wing in August, so the writer has worked out the flying temperatures for August at four points, Yuma, Arizona, St. Louis, Missouri, St. Paul, Minnesota, and Sitka, Alaska. Each of these is representative of the flying conditions for a restricted group of Æshnas. The Yuma temperatures apply to *jalapensis*, *multicolor* and *arida*. The St. Louis

[5] Merriam, "Life and Crop Zones of the United States," Bull. U. S. Biol. Survey, No. 10, pp. 1–79, 1898.

temperatures apply to *constricta*, which is the only species broadly distributed across the central states, while the St. Paul temperatures apply to a series of several species such as *interna*, *canadensis*, *umbrosa*, etc. Under the Sitka temperatures we find *sitkensis*, *subarctica* and *septentrionalis*. Such species as *palmata* and *constricta* are found in two or three of these zones.

To define the flying temperatures the mean maximum and mean minimum day temperatures for August have been taken from the Weather Bureau Report for the above stations.[6] Ten degrees was then added to the mean maximum day temperature to give the approximate maximum sun temperature, which is the temperature to which the local dragonflies react positively, while the mean minimum day temperature remained unchanged, as it is a shade temperature to which the local dragonflies react negatively. Thus these temperatures show roughly the range of day temperatures which the species of *Æshna* meet during their flight season in each of the general zones represented by the temperature records. These records for August are as follows:

	Mean Maximum in the Sun.	Mean Minimum in the Shade.
Yuma, Arizona	114°	77°
St. Louis, Missouri	96°	69°
St. Paul, Minnesota	90°	60°
Sitka, Alaska	72°	45°

From the above it is obvious that the temperatures during flight do differ greatly for the various species of *Æshna*. However our observations indicate that all lowland Æshnas are always positive to heat so that the higher the temperature, the greater the activity of the insect. Hine's observations[7] on Kadiak Island were that *palmata* was most active on the warmest days. Walker's observations[8] on the Canadian species are that increased temperatures always increased the speed of Æshnas but his observations do not include temperatures above 90°. Somewhere above 90° there may be a limit for this increase of

[6] "Climatology of the United States," Bull. 2, 1906. Temperatures for Yuma, St. Louis and St. Paul. *Monthly Weather Review*, Dec., 1898, p. 549. Temperature for Sitka.

[7] In conversation with the writer.

[8] Walker, p. 33. "The North American Dragonflies of the Genus *Æshna*," Univ. of Toronto, Biol. Studies, Biol. Series, No. 11, 1912.

speed as the temperature rises, which limit may be actually reached by some of the southern species on very warm days. The writer [9] has made one interesting observation in this regard. *California* and *multicolor* on very hot days when the temperature is from 100° to 105° take frequent rests, by hanging up in the shade every few minutes. This was noted in the Yakima desert at Sunnyside, Washington. Apparently, for these at least, somewhere in the higher nineties a temperature is reached above which increase is depressing and no longer stimulating. However we can safely say that for all lowland species of *Æshna* an increase of temperature up to 95° increases activity so that all are equally positively thermotropic.

The thermal distribution of the four groups of species outlined above must then be conditioned through some indirect check on the life history. Some temperature condition of the water for the nymph or the developing egg may be the limiting factor rather than the flying temperature for the imago. Undoubtedly individuals of each species continually fly beyond the limits of the optimum habitat but the offspring of such do not survive or we would have a spreading species. This constant pressure of the species of dragonflies into surrounding but unsuitable habitats was worked out by the writer [10] on Put-in-Bay Odonata in 1922. Until we know the life histories of the various species in minute detail we will not be able to define all of these limiting factors except as we stumble onto them accidentally.

A further conclusion appears indicated from this study. As all Æshnas, except *nevadensis*, have one type of reaction to temperature in the imago, and all the Ephemeras, except *guttulata*, have one type of reaction to light and the reactions of these two odd species are just the reverse of the other species in their respective genera, we may conclude that a given type of insect nervous system can be completely reversed from its usual reaction more easily than it can be modified in a lesser degree. This would appear logical from the theoretical grounds of the mechanics of the nervous system. Because of its minute size, the insect nervous system is characterized by the relatively

[9] Walker, see ref. 8, p. 33.

[10] Kennedy, "The Ecological Relationships of the Dragonflies of the Bass Islands of Lake Erie," *Ecology*, Vol. III., pp. 325–336, 1922.

small number of units (either cells or combinations of cells) that comprise it. Such simple nervous systems obviously cannot produce the extended series of finely graduated reactions that are possible to a more complicated type of system. On the other hand the mechanism is already present for the reversal of any particular reaction. So a complete reversal of a generic reaction which puts the species entirely outside of the normal generic habitat, is more likely than the slight modification necessary to put it into a near but only slightly different habitat in which it would be held by only a slight gradation of the general reaction.

At first thought, if it is easier to have a reversed reaction in the insect nervous system than to have a graduated reaction, one might think that the genus would fly apart as to any unity of environment, so that each species would have a habitat strikingly different from that of each other species, that there would be no such thing as a generic habitat. Observation shows that this is not so. Species are superimposed and habitats overlap in a most confusing manner and in any large genus there is usually an easily recognized generic type of habitat. So far not enough experimental work on behavior has been carried out to determine if the species of any one genus of insects are distributed by graduated reactions to one type of stimulus. However an analysis of some of the factors of the problem appear to indicate that such a distribution, for instance as the species of *Æshna*, show in a series of temperature gradations, may not be due to a slight difference in reaction to a specific stimulus, but may be merely an apparent series each species of which is held in its particular zone by some different positive or negative reaction to any one of a variety of stimuli.

An insect with incomplete metamorphosis passes through two stages, nymph and adult, in each of which it may pass through several physiological stages of development. The nymph has at least two, its feeding stage and its quiescent preëmergence stage. The imago has at least five stages, the teneral, the sexually mature stage, which can be divided into periods of hunger, periods of sexual lust, periods of egg-laying, and finally the stage of senility. The last need not be considered in a problem of species distribution as it is beyond and outside the genetic cycle. In each of these stages several stimuli control the individual,

the reaction to anyone of which may be the factor that limits the species to its particular environment. If we consider only ten of the more easily recognized tropisms then in the six physiological stages enumerated above we have one hundred and twenty positive and negative possibilities for a reaction to an individual stimulus that might limit the species to a specific habitat. The problem is really much more complicated than this as many more reactions enter and all are more or less conditioned by morphological factors. So there is no difficulty at all in accounting for a great variety of factors, any one of which may limit the species to a rather narrow habitat.

In building the conception in the student's mind of the group of habitats occupied by all the species of a genus the mind automatically picks the most obvious habitat characteristic that is common to all the habitats under consideration. If this habitat characteristic, such as flying temperature for *Æshnas*, varies from habitat to habitat the mind automatically considers each species delicately adjusted to the exact degree of this variation in its specific habitat. This may not be true at all. And the factor which limits each species may not be the obvious one but some inconspicuous factor and it may be in each species in the genus a factor from a different category. It may be a temperature factor in one, a moisture factor in another, a chemical factor in a third species, etc. Thus graduated generic factors may largely be a compound figment of the mind of the observer.

THE INDIVIDUALITY OF THE GERM-NUCLEI DURING THE CLEAVAGE OF THE FERTILIZED EGG OF THE ROTIFER, *ASPLANCHNA INTERMEDIA*.[1]

BARBARA WIGGENHORN,

DAVID D. WHITNEY.

Recently while studying sections of the rotifer, *Asplanchna intermedia* (Hudson and Goss), double nuclei were discovered in the fertilized eggs while only one nucleus was seen in the parthenogenetic eggs. All of the cells in the early cleavage stages showed the two nuclei but in the later stages fewer and fewer cells contained two nuclei. It was decided to study the sections in an attempt to determine how late in the embryonic development the two nuclei remain distinct.

This species produces small parthenogenetic eggs developing into males, larger parthenogenetic eggs developing into females and large fertilized eggs which also develop into females. Both kinds of parthenogenetic eggs may be distinguished readily by their very thin covering membrane. It appears to be no thicker than the covering membrane of the blastomeres of an embryo. (Fig. 1, *A–G*). The covering of the fertilized egg is many times thicker and also shows a different structure than that of the parthenogenetic eggs. By noting the covering membranes alone one can distinguish these two kinds of eggs at a glance. (Fig. 1, *A*, *D*, *H*). No observer has ever found a parthenogenetic egg with a thick shell in this species.

When the male parthenogenetic egg is fertilized it becomes larger, develops this thicker characteristic shell and is popularly known as the winter egg. The origin of both of these eggs is identical but their mature appearance depends upon whether they are fertilized or not. They both produce two polar bodies while the female parthenogenetic egg only produces one polar body.

[1] Studies from the Zoological Laboratory, The University of Nebraska, No. 142.

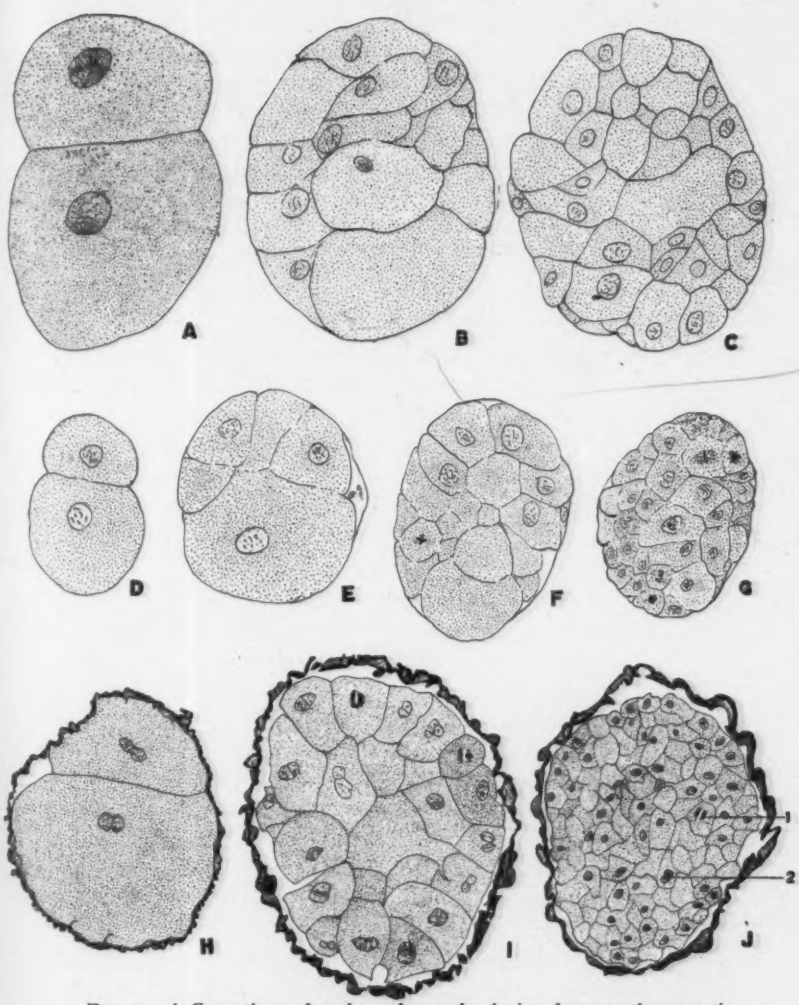

FIG. 1. *A–C*, sections of early embryos developing from parthenogenetic female-producing eggs showing one nucleus in the cells; *D–G*, sections of early embryos developing from parthenogenetic male-producing eggs showing one nucleus in the cells; *H–J*, sections from embryos developing from fertilized eggs showing two nuclei in some of the cells.

As this form of rotifer is ovivivíparous and the females frequently carry from 1–10 developing embryos and young it was found practical to fix entire individuals in the killing fluids. Flemming's strong solution for about one hour was used. Then the material was bleached, dehydrated and imbedded in clove oil and paraffin. Large numbers of individuals were sectioned in masses, cutting sections 5 microns in thickness and the favorable sections studied. These animals were raised in great numbers in laboratory cultures in weak horse-manure solutions. The figures were outlined by means of a camera lucida with a magnification of about 430 diameters and the details filled in free hand.

As it is the male parthenogenetic egg that can be fertilized and changed into a larger and thicker shelled egg it is of some interest to compare the nuclei of the cells in the embryos developing from these eggs without fertilization and with fertilization. For a complete comparison the female parthenogenetic egg has been included.

Each cell of the embryo developing from both kinds of the parthenogenetic eggs contains only one nucleus regardless of whether it is in the 2-cell stage or in later stages of a larger number of cells (Fig. 1, *A–G*). Many hundreds of sectioned embryos have been observed in the various celled stages and not one has been found whose cells contained more than one nucleus.

A few somatic cells were found in which the chromosomes could be counted fairly accurately in the embryos developing from both kinds of the parthenogenetic eggs. The small male embryos developing from the male parthenogenetic egg showed the haploid number of about 25 and the larger female embryo developing from the female parthenogenetic egg showed the diploid number of about 51 chromosomes (Fig. 2, *A–B*). Although the chromosomes are very small and somewhat crowded together their exact number is reasonably certain. Tauson working on this same species has concluded the haploid number in the mature male parthenogenetic egg to be 24 and the diploid number of 48 in the female parthenogenetic egg.

In the early embryos developing from the fertilized eggs two distinct nuclei appear in each cell. These nuclei usually appear apposed to each other but clearly distinct (Fig. 1, *H*). In later stages only some of the cells show the two nuclei and as the

embryo becomes older and is comprised of many more cells very few of the cells are found containing two nuclei, as for instance about the 250-cell stage only two such cells were found in a section (Fig. 1, *I–J*). Beyond that stage no cells have been found containing two nuclei.

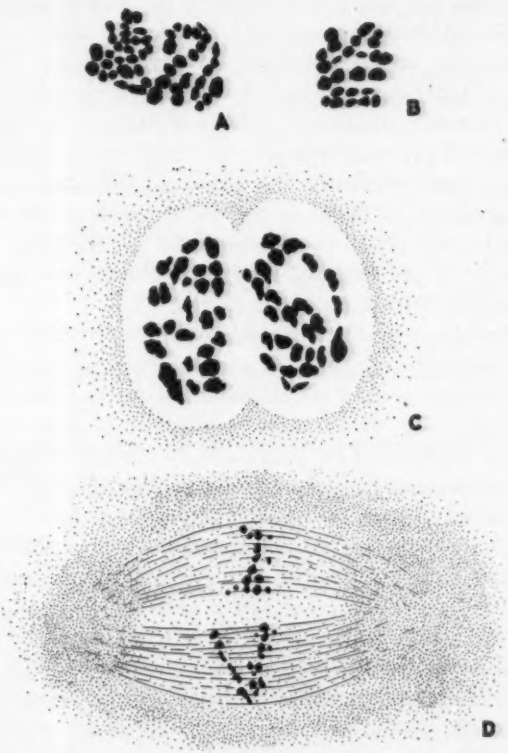

FIG. 2. *A*, somatic cell of a female embryo developing from a parthenogenetic egg showing 51 chromosomes; *B*, somatic cell of a male developing from a parthenogenetic egg showing 25 chromosomes; *C*, the two nuclei of a matured and fertilized egg showing 26 chromosomes in each nucleus; *D*, a double spindle from a somatic cell of an embryo developing from a fertilized egg. Drawings somewhat diagrammatic because of minute size of chromosomes.

Mitotic figures occur showing double spindles which seem to indicate that the two nuclei divided independently of each other in cell division (Fig. 2, *D*). Why this occurs only in the cells in

the early stages of the embryo is not determined. Perhaps as the cells become more numerous and consequently smaller the size ratio between nucleus and cytoplasm is so altered that there is only room for one mitotic figure and consequently both nuclei are crowded into one fused nucleus.

A section was found of a fertilized egg in the one-cell stage in which the chromosomes could be counted fairly accurately. Each nucleus seemed to have 26 chromosomes. This fact clearly differentiates it from either of the parthenogenetic eggs. It also furnishes additional evidence to support the claim that the fertilized egg was originally the male parthenogenetic egg which has been entered by the sperm. The haploid number of 26 chromosomes is found in the mature male parthenogenetic egg and in each of the two nuclei of the fertilized egg. One of these nuclei in the fertilized egg is undoubtedly the egg nucleus and the other is from the sperm (Fig. 2, *C*).

Further observations of the sections of the fertilized eggs show that in the first cleavage each nucleus gives rise to two groups of chromosomes each of which passes separately to the daughter nuclei (Fig. 2, *D*). During the ensuing resting stage each germ nucleus is represented by a structurally distinct vesicle. Thus the separateness of the germ nuclei is maintained throughout the entire nuclear cycle. In mitosis there seem to be two spindles each with its distinct set of chromosomes which separate regardless of each other. Probably each group of chromosomes splits into halves, and a maternal and a paternal group go to each end of the double spindle, so that each daughter cell receives two sets of chromosomes around which separate walls are formed, and a maternal and a paternal vesicular nucleus appear.

The double nuclei have been found up as far as about the 250-cell stage. In one section of an embryo there were 103 visible cells and among these two had double nuclei (Fig. 1, *J* 1–2).

Two distinct nuclei in fertilized eggs and their developing embryos have been observed in other forms by various other workers. Mark observed this phenomenon in *Limax*, Van Beneden in *Ascaris*, Häcker in *Cyclops*, Conklin in *Crepidula*, Beard in *Raja batis*, Smith in *Cryptobranchus allegheniensis* and other workers have seen indications of it in other forms. No

one has observed this in rotifers previously nor have the two nuclei been seen in as late stages as in this species of rotifer.

Summary: It has been found in *Asplanchna intermedia* that the germ nuclei in the fertilized egg do not fuse in the early cleavage stages, but each gives rise to two sets of about 26 chromosomes, one set of which pass into each of the daughter nuclei. Each nucleus is a structurally distinct vesicle.

In later cleavage stages the nuclei become fused so that in about the 250-cell embryo only a very few show the two nuclei in one cell.

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THE RELATION OF CARBON DIOXIDE TO SPONTANEOUS MOVEMENTS IN THE LARVÆ OF *OPSANUS TAU.*

HENRY C. TRACY.[1]

Spontaneous movements in embryos have been reported in the literature since the time of William Harvey (1651). Such movements have been observed in many groups of animals, and their occurrence is probably a universal phenomenon.[2]

More or less definite statements as to a relation between embryonic movements and respiratory conditions, have appeared several times in the literature.

Balfour ('76) says: "The band [of longitudinal striated muscles] developed at this stage [*H*] appears to be a special formation which has arisen through the action of natural selection, to enable the embryo to meet its respiratory requirements, by continually moving about, and so subjecting its body to fresh oxydizing influences; and as such affords an interesting example of an important structure acquired during and for embryonic life."

Ahlfeld ('05) showed the existence of rhythmic movements in the human fetus during the second half of pregnancy, manifested by rhythmic undulations of the maternal abdominal wall. These movements were near the rate of respiration in the newborn and were interpreted as preliminary respiratory movements.

Sarwey ('15) states that there is an increase in the strength and frequency of fœtal movements at the beginning of asphyxia and shortly before death.

Minkowski ('21) says: "On admet generalment que l'asphyxie de la mere mene a une augmentation des mouvements fœtaux, l'asphyxie directe du fœtus pourrait donc egalement le faire. Le manque d'oxygene agirait alors comme une excitation inter-

[1] Contribution from the laboratory of the United States Bureau of Fisheries, Woods Hole, Mass., and the Department of Anatomy, University of Kansas.

[2] References to the literature on these movements may be found in Preyer, '85; Munkowski, '21, and Wintrebert, '20.

ieure provenant du sang, et determinerait ou favoriserait du moins les mouvements du fœtus."

Wintrebert ('14) states that in the trout the embryonic contractions are more frequent and lively in a medium containing CO_2 than in one containing oxygen. He considers ('20, pp. 450), these movements favorable to respiration, excretion, and circulation. He also states that lack of oxygen depresses the movement (in Selachians).[1]

Experimenting with cat fetuses eight to nine centimeters long, T. Graham Brown ('14) observed that rhythmic alternating movements of the limbs (interpreted as progression movements) arose spontaneously and could also be produced by asphyxia resulting from pressure on the umbilical cord.

In adult animals, the relation between the respiratory center and CO_2 has long been known, even though the exact mechanism is still in doubt. Stimulating effects of asphyxial conditions on blood pressure and motor mechanisms through the spinal cord have been reported (Mathison, '10 and '11).

A. D. Waller ('96) found an increased irritability in nerve fibers treated with CO_2, the effect varying with different concentrations. Roaf ('12) showed that the rate of branchial movements in fish showed a positive correlation with the H-ion concentration and with the concentration of CO_2; the appendages of barnacles showed a negative correlation to these conditions.

These observations and other well known physiological studies indicate that stimulation by metabolites occurs in many different varieties of contractile and transmissive mechanisms; it, therefore, may be expected that alteration of body fluids with respect to respiratory conditions may have some relation to the endogenous movements of embryos, and in fact it is possible that changes in such conditions may be the most important factors in the production of these movements.

The experiments reported in this paper were undertaken in order to test the effect of different concentrations of CO_2 in the sea water on the spontaneous movements of larvæ of *Opsanus tau*.

The yolk sac, with the network of capillaries over its surface,

[1] So far as his descriptions go, the depression of movements which he observed appears to have taken place under asphyxial conditions and were probably due to the narcotic action of a considerable excess of CO_2 (Wintrebert, '20, pp. 324 and 328).

furnishes favorable conditions for an interchange of substances between the sea water and the blood of the larvæ and probably acts as a respiratory organ before the branchial mechanism becomes functional. It is, therefore, comparatively easy for purposes of experiment to change certain chemical characteristics of the body fluids of the larva by altering the character of the sea water around it.

Opsanus tau (toad fish) is an inactive bottom fish which inhabits shallow, sheltered water. The eggs are found in such localities, fertilized and in the process of development, attached to the under-side of sticks, stones, tin cans, etc. The objects to which the eggs are attached may be brought into the laboratory where they develop in an apparently normal manner if furnished with a constant supply of fresh sea water. The eggs hatch in 3 or 4 weeks; the yolk sac, however, remains attached; about three weeks later, the larva has absorbed nearly all the yolk; it then becomes loosened from its attachment and swims free.

In this species the first observable movement is the heart beat which begins in specimens of 12 to 14 somites. The earliest stage at which muscular movements of the body were observed was in a specimen about 19 somites. Movements appear in the first few somites (2 to 4 or 5); the somites contract slowly and apparently simultaneously, causing a lateral bending of the anterior body region.

These movements take place singly and at irregular intervals, often with several minutes between and with no apparent relation between successive movements. Responses to external stimuli, however, do not take place until a much later period, in fact, not until after hatching. There seems, therefore, no question that these early movements are the result of changes in the internal conditions.

Soon, however, there appears a slight tendency for movements to appear in groups; this tendency increases until at the time of hatching, in addition to occasional single bendings of the body, right and left alternating coils of the body often take place very rapidly for a brief interval, producing a kind of vibratory or "fluttering" movement of the whole body. These "fluttering" movements become more and more predominant during the larval stage. When the free swimming period begins the "flut-

tering" movements of the body which alternate with irregular intervals of rest have now become regular undulating vibrations which cause short bursts of swimming movements of the fish through the water.

It is evident that the spontaneous movements of the embryo are gradually elaborated into the swimming movements of the free larva. Similar bursts of motion at the end of considerable intervals of rest on the bottom are characteristic of the adult throughout its sluggish existence. In the other teleosts which I have observed (*Fundulus* and *Tautogalabus*), the embryonic movements occur with much greater frequency and with only very brief intervals between; during development these movements become merged into the continuous type of movements characteristic of fishes of pelagic habits. It would appear that the activity habits of these animals are not widely different at any stage of their existence, and are determined by some internal physiological mechanism, the earliest expression of which is found in the spontaneous movements of the embryo.

Spontaneous movements of the mandible and branchial mechanism begin soon after hatching; they are, at first, slight and irregular, but gradually, in the course of about 5 days, they develop into the respiratory rhythm. Exteroceptive and proprioceptive reaction mechanisms do not respond to external stimuli at the time of hatching but gradually become functional during the larval period.

Preliminary experiments were carried out in the summer of 1922 at the Marine Biological Laboratory at Woods Hole.[1] Newly hatched larvæ were exposed to sea water from which all the gases were removed by passing hydrogen for an hour and a half through sea water which had been previously boiled.

Of 13 specimens, under these conditions, 11 showed a decrease in spontaneous movements. In another set of experiments 8 larvæ were exposed to sea water in which a number of *Fundulus* had been allowed to remain until they showed signs of asphyxiation; of these, 6 specimens showed a decided increase in spontaneous movements. In another experiment, 6 specimens were placed in KCN $n/1000$ in sea water. In all of these specimens,

[1] The research room at the Marine Biological Laboratory was supplied me from the Graduate Research Fund of the University of Kansas.

the movements were immediately stimulated to such an extent that they were nearly continuous during the first 15 minutes; they slowed down gradually, reaching about normal at the end of the first hour; during the next following 15 minutes, the movements ceased entirely. The results of these experiments show a close resemblance to reactions which would have been expected from a respiratory mechanism under similar conditions.

During the summer of 1923 at the laboratory of the United States Bureau of Fisheries,[1] at Woods Hole, I attempted to carry out more accurate experiments by subjecting *Opsanus* larvæ to sea water of known definite partial pressures of CO_2. For suggesting this method I gratefully acknowledge my indebtedness to Doctor Homer W. Smith. Details of the method have since been published (Smith and Clowes, '24).

Methods.

The method of experiment consisted essentially in adding different percentages of HCL $n/20$ to the sea water in which the larvæ were placed. The changes in CO_2 partial pressure, and the H-ion concentration produced by this means are stated in the tables.

In applying this method, the following sources of error had to be taken into account:

(1) Variation in frequency and duration of the spontaneous movements in different individuals, and in the same individual at different times.

(2) Temperature changes.

(3) Stimulation by manipulation and handling of the embryo previous to the beginning of each experiment; previous observations had shown that manipulation of the yolk sac which results from handling of the larvæ, pressure of flowing water, etc., may cause an increased movement even though the larvæ do not respond to direct tactile stimulation.

[1] In connection with laboratory arrangements in the conduct of these experiments I am greatly indebted to Dr. R. E. Coker, Director of the Laboratories of the Woods Hole Station of the U. S. Bureau of Fisheries.

(4) Stimulation of movements which might result from the shaking the bottle containing the specimens, after the addition of the acid to the sea water.

To control these sources of error, the following routine procedure was adopted. The larvæ were taken from the stock in the aquarium, scraped off from their attachment, and placed in a 250 cc. flask of fresh sea water at 25° C., and allowed to remain undisturbed from one half hour to an hour. The bottle containing the larvæ was then shaken a definite number of times (20 uniform back-and-forth movements). This procedure served as a control for the shaking after addition of the acid; several experiments seemed to show that stirring of the sea water in this manner had no appreciable effect on the movements of the larvæ, but nevertheless, at the beginning of each record of normal movements the bottle was shaken as described. The movements of the larvæ were then recorded for a period varying from 15 minutes to one half hour; this furnished the record of the movements under normal conditions of each individual at 25° C. At the end of this time, a little sea water was poured out of the bottle, the proper amount of HCL $n/20$ added and the contents of the bottle made up to exactly 250 cc. with sea water and the stopper inserted. These operations were carried out as rapidly as possible in order to prevent the escape of CO_2 into the air. The bottle was then stirred (as described above for the normal control) in order to secure as rapidly as possible a uniform CO_2 pressure and H-ion concentration throughout the water in the bottle. The movements were then recorded, usually for 30 minutes to one hour. At the close of this period, the acidulated sea water was poured off, and replaced with fresh sea water of the same temperature. The slight agitation of the larvæ which was unavoidable in pouring off the acidulated water is probably a sufficient control for the stirring at the beginning of the previous steps in the experiment. A record of the movements was then made.

In making the record, the time, character and extent of the movements were noted. A single coil to one side was taken as the unit. The number of separate movements can be recorded in this way with considerable accuracy; but in the case of movements following each other in rapid succession, as in the

"fluttering" movements mentioned above, a somewhat arbitrary method is necessary. These "flutters" were assigned a value of 10, which seems about the minimum estimate of their value. At the free swimming stage these "flutters" sometimes continue several seconds which, of course, cause an extended swimming movement; a value of 10 for each second was given for such movements. The results thus arrived at are partly based on estimates and hence cannot be exact. But they will serve to give the general order of magnitude of the movements of the larvæ under different conditions. Most of these movements are undoubtedly greater than the minimum assigned value; hence the error favors a negative result. Since the method of estimation of numerical value was the same for the same larva under both control and experimental conditions, errors due to the method of recording tend to neutralize each other.

The records of each experiment were afterward transferred to chart records by recording the total numerical value of the movements for each minute on cross-section paper. The records of all specimens for each concentration of CO_2 were then averaged for each minute; then, in order to smooth the curves somewhat, averages for each two minutes were taken and recorded as shown on the charts accompanying this paper.

Observations were made at two periods of the larval development:

(1) Within the first five or six days after hatching and before external respiration had begun (larvæ about 6 or 7 mm. in length). At this stage there is no response to light, rotation, jar, or vibrations and little or none to tactile stimuli.

(2) At the end of the larval period when the yolk sac is nearly absorbed, and the larvæ are on the point of swimming free (18–20 mm. in length). All the reaction mechanisms, as far as known, are now functionally developed.

The behavior of the respiratory mechanism in the free swimming larvæ (second period) was observed under the different conditions of CO_2 and the record superimposed on the charts recording the body movements. The time in seconds necessary for the completion of 10 respiratory movements was taken with the stop watch for each individual (whenever possible the

average of 3 observations for each minute), and the average of all the individuals for each concentration of CO_2 was indicated on the charts for each two minutes by the number of spaces above the base line.

Results.

In the first stage 3 or 4 specimens can be studied at the same time in one experiment; but in the free swimming stage only one specimen can be recorded at a time. The breeding period of this species is brief, hence, the number of individual records obtainable is not as large as is desirable, but when the averages are brought together the conclusions stated below seem justified. But the number is insufficient to give a smooth contour to a curve indicating the effect of any given concentration with respect to the time of exposure or to state in an exact manner the effect of different concentrations.

The chart records indicate the following relation between changes in the partial pressure of CO_2 and the body movements and respiratory rate.

A. Body movements.

1. The movements in each group show an increase during the first few minutes following an increase in the concentration of CO_2.
2. When the specimens in the water containing the higher concentration of CO_2 are returned to normal sea water, the movements in each group are depressed considerably below the normal. This depression is very pronounced during the first 10 minutes and gradually approaches the normal during the 30 minute period.
3. The intensity of the reaction varies with the increase in CO_2, up to the middle range of concentration; above this the intensity of stimulation decreases with the increase of CO_2 concentration.
4. The reaction to increased CO_2 is less intense but of greater duration in the earlier than in the later stage. On returning to normal sea water the depression of movement is less in the earlier than in the later stage and recovery is slower.

The different effects in the two stages are not easy to explain.

They might possibly be due to the presence of the large yolk sac in the younger stage. The mass of the larva at hatching is considerably less than that of the yolk; the yolk then must act as a "buffer" and the increased CO_2 is "soaked up" from the blood by the yolk substance inside the vitelline net work nearly as rapidly as the blood absorbs it from the water outside. The increase in concentration in the body fluids of the larvæ is, therefore, relatively slight at first and increases slowly until the yolk substance approaches equilibrium with the sea water relative to the CO_2 concentration. It is only in the high concentrations that this can take place during the first 10 minute period. In returning to normal sea water the blood begins to absorb the CO_2 back from the yolk and hence the depression period is pronounced.

At the beginning of the free swimming stage the amount of yolk remaining is very slight, and in the gills active interchange of gaseous substance is taking place directly between the sea water and the body fluids of the larva; the effect of increasing the concentration of CO_2 in the sea water is, therefore, almost instantaneous and hence the stimulation rapidly reaches its maximum, followed quickly by the depression due to effects of excessive CO_2. The recovery in the normal sea water is rapid on account of the rapid attainment of equilibrium between the inside and outside of the larva through the branchial system.

It is, however, difficult to understand why it should require so long a time for the yolk substances to take up the CO_2. According to the combined record of the newly hatched larvæ (Chart XII.) the heightened reaction lasts about 25 minutes, but only 10 minutes in the free swimming (Chart XIII.).

There are, of course, other differences between the two stages. In the first there is a relative preponderance of undifferentiated tissue, as compared with the later stage. The chemical relations of young tissue must be different from older. The larvæ will endure asphyxial conditions better than the adult (see p. 19). Anaërobic respiration is known in the young of other forms. Susceptibility to strychnine is less in young than in adults (Schwartze, '22). Apparently, young tissue has greater adjustment capacities to factors affecting a fundamental activity like metabolism than older tissue.

It must be stated that there are a few aberrant specimens which fail to react in the usual manner. The number and incidence of these are shown in the tables. Some of these exceptions are only apparent, since most specimens show movement either while the bottle containing them is being shaken or in the interval (a minute or less) immediately afterward, before the record can begin. These movements, of course, do not appear in the record. Also, there seem to be more aberrant specimens in the higher concentrations because of the stronger narcotic action of CO_2 and its more rapid onset. Some are perhaps pathological in spite of careful effort to select only healthy specimens; there is a large mortality under laboratory conditions, perhaps 50 per cent. during the larval period. Most of these exceptional specimens are probably thus explained; whether all can thus be accounted for, is uncertain; there may be physiological conditions in which an increased CO_2 may produce a depression immediately instead of stimulation.

B. Rate of Respiratory Movements.

In the lower ranges of concentration of CO_2, the respiratory rate is faster during the period of exposure (Charts VI., VII., VIII.). The middle ranges of CO_2 concentration (Charts IX. and X.) appear to result in a balance between the stimulating and depressant effects. In the higher ranges (Chart XI.) after a short period of stimulation, the rate falls considerably below the normal. In the extreme concentrations (addition of 3.5 per cent.–4 per cent. acid to the sea water—Chart XIV.) the respiration becomes irregular, then of the Cheyne-Stokes type; after a time it gradually improves and becomes regular, though proceding at a somewhat slower rate than in the normal. Reuss ('10) has shown the stimulating effect on the respiratory rate of moderate concentration of CO_2 in adult fish and the depressant effect of higher concentrations. There is, therefore, a tendency for these higher concentrations to break down the respiratory system; the capacity for recovery is probably dependent on some secondary reserve mechanism. This capacity for recovery seems not to be possessed by the spontaneous movements.

On return to normal sea water, the effect of the lower concentrations (Charts VI., VII.) is the same on the respiratory rate as on the movements, that is, a depression below normal;

in the case of the higher concentrations, the respiratory rate rises above the normal on return to normal sea water.

Obviously there is a close similarity between the effect of CO_2 on the rate of movements of the body and rate of respiratory movements in the lower concentrations; in the higher concentrations the respiratory mechanism has a compensatory capacity both during exposure and in recovery afterward, which is not possessed by the motility mechanism. Apparently, then, the regulation of respiration and motility at ordinary concentrations of CO_2 takes place through similar mechanisms; in high concentrations, the respiratory system can bring into play secondary reserve mechanisms, which appears to be beyond the capacity of the system controlling body movements.

The resistance of these larvæ to asphyxiating conditions is very great. One of these laryæ was left over night in a bottle containing the highest concentration of CO_2; the next day the specimen lay motionless on the bottom of the bottle with the respiratory rate about the same as shown on the preceding day; on putting the specimen into fresh sea water the respiratory rate quickly jumped above the normal, the body movements approached the normal in 12 to 15 minutes. Larvæ placed in water from asphyxiated *Fundulus* (which causes acute symptoms in half grown adult toad fish) apparently suffered no inconvenience; in boiled sea water, through which hydrogen had been bubbled for one and a half hours, the early larvæ show no effects during the first hour and a half (aside from reduction of body movements) although oxygen was entirely absent. Evidently anaërobic respiration is possible for a considerable time at least, in these embryos.

Stockard ('21, p. 173) indicated the probability that double and abnormal embryos in fishes may be produced by asphyxial conditions at certain stages. In the toad fish I have found only one double larva, although in the course of several seasons I have examined several thousand specimens. This high degree of resistance to asphyxial conditions is no doubt correlated with the crowded stagnant condition of the water in which this species passes its embryonic and larval life.

That there is some definite relation between spontaneous movements and respiratory movements is shown by the reduction

in the rate of respiratory movements which seems always to exist immediately after each spontaneous movement (Chart XV.). At the end of each movement the branchial apparatus is motionless for a few seconds; it begins activity very slowly, and gradually resumes the normal rate in 10 to 15 seconds. This phenomenon may be due to acapnia induced by the rush of water through the gills while the larva is in motion; furthermore, if it is the production of metabolites which stimulates the body movements, the same substances would probably increase the volume of blood flowing through the branchial vessels and thus favor the washing out of CO_2 from the blood; a brief inactivity of the respiratory mechanism then ensues, due to the lack of stimulation.

Speculation as to the details of the mechanism by which embryonic movements are stimulated and depressed under the various conditions described in this paper seems premature until the relation between specific effect of CO_2 and variations in the H-ion concentration has been experimentally determined. Experiments with KCN, with different concentrations of CO_2, and with CO_2 removed, indicate that essentially similar mechanisms regulate respiratory and body movements, except for some accessory reserve system which exists in the case of respiratory movements.

The relation of different concentrations of CO_2 to the amount of motility must be a significant factor in determining the migrations and habitat of fishes. Shelford, Wells and Powers, (Shelford, '23) in their work on the relation of H-ion gradients to the movement of fishes apparently did not study the mechanism of the reactions. The results which these writers report are perhaps due to the direct effects of H-ion changes in the body fluids on the primitive neuro-muscular system, or, in other words, to variations in the rate of the endogenous, "spontaneous" movements conditioned by alterations in the amount of CO_2 or H-ions in the surrounding medium. According to this view, the optimum H-ion concentration is automatically determined by the effect of different parts of the acidity gradient on the endogenous movements, and not to any "choice" or "selection" on the part of the fish.

A question of broader significance is raised by these experi-

ments since the results suggest that endogenous movements may be stimulated by variations, local as well as general, in the concentration level of metabolites which result from differences in the metabolism rate in different regions of the embryo. Jacobs ('22, p. 25) has made a similar suggestion regarding the production of pseudopods in *Amœba*.

Summary and Conclusions.

1. Newly hatched and free swimming larvæ of toad fish (*Opsanus tau*) were subjected to different concentrations of CO_2 produced by additions of different percentages of HCL $n/20$ to sea water.

2. Increased concentration of CO_2 is followed by an increase in the endogenous (spontaneous) body movements in both stages; in newly hatched larvæ the reaction of CO_2 is less intense but of greater duration (average about 25 minutes) than in free swimming larvæ (average about 10 minutes).

3. On return to normal sea water from the higher concentration of CO_2, the frequency of the body movements is depressed below the normal; the depression is less in the newly hatched larvæ than in the free swimmer and the recovery is slower.

4. In the lower ranges of CO_2 concentration, the body movements (in both stages) and the rate of respiratory movements (free swimmers) vary with the increase in CO_2; in the higher ranges the body movements, after a period of stimulation, remain depressed far below the normal, while the respiratory movements, after a brief stimulation followed by depression and irregular rythm, recover and proceed regularly at a little below the normal rate.

5. On return to normal sea water, the body movements for all concentrations of CO_2 remain depressed (about 30 minutes for the free swimmers, longer for the newly hatched larvæ); the rate of respiratory movements is below the normal for the lower concentrations, but is increased above the normal on return from higher concentrations.

6. Respiratory movements and spontaneous body movements react similarly to the lower concentrations of CO_2 and hence their regulation probably takes place through a similar mechanism; at the higher concentrations, the respiratory system

appears to bring into play a secondary reserve mechanism which gives it a compensatory capacity not possessed by the neuromuscular system through which body movements are produced.

7. Toad fish larvæ are much more resistant to asphyxial conditions than adults.

8. It is suggested that the migration of fishes in a H-ion gradient is probably conditioned by the effect of acid substances on the endogenous body movements.

9. It is suggested that stimulation by variation in the concentration level of metabolites produced inside the body may be the source of endogenous (spontaneous) movements.

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Explanation of Tables and Charts.

Charts I. to V. are records of specimens just hatched, before the beginning of respiration.

Charts VI. to XI. are records of specimens at the beginning of the free swimming stage.

Chart XII. is the combined record of the movements of the just hatched larvæ under all concentrations of CO_2.

Chart XIII. is the combined record of the movements and respiration rate of the free swimming larvae under all concentrations of CO_2.

Two minute spaces are marked off on the horizontal base line: height of the line indicates the average amount of movement (estimated as described on p. 10) made in each minute of the two-minute period by the specimens in each concentration of CO_2. The average amount of movement each minute under normal conditions for the time observed (15 minutes to 1 hour) is indicated by the arrow at the beginning of each chart, marked A. N. M. The arrow marked A. M. R. indicates the average movement per minute when the larvæ are returned from the acidulated water back to the normal.

In Charts VI.–XI. and XIII. the respiration record of the free swimmers is superimposed on the movement record (respiration not being established in the just hatched larvæ). The height of the short horizontal line in each two minute space indicates the number of seconds (usually the average of three observations in each minute) taken for 10 respiratory movements (average for all the specimens of each concentration of CO_2). Hence a drop in the line means increased respiratory rate, and vice versa. The observed normal rate (average for each minute) is indicated at the beginning of each chart by an arrow marked A. N. R.

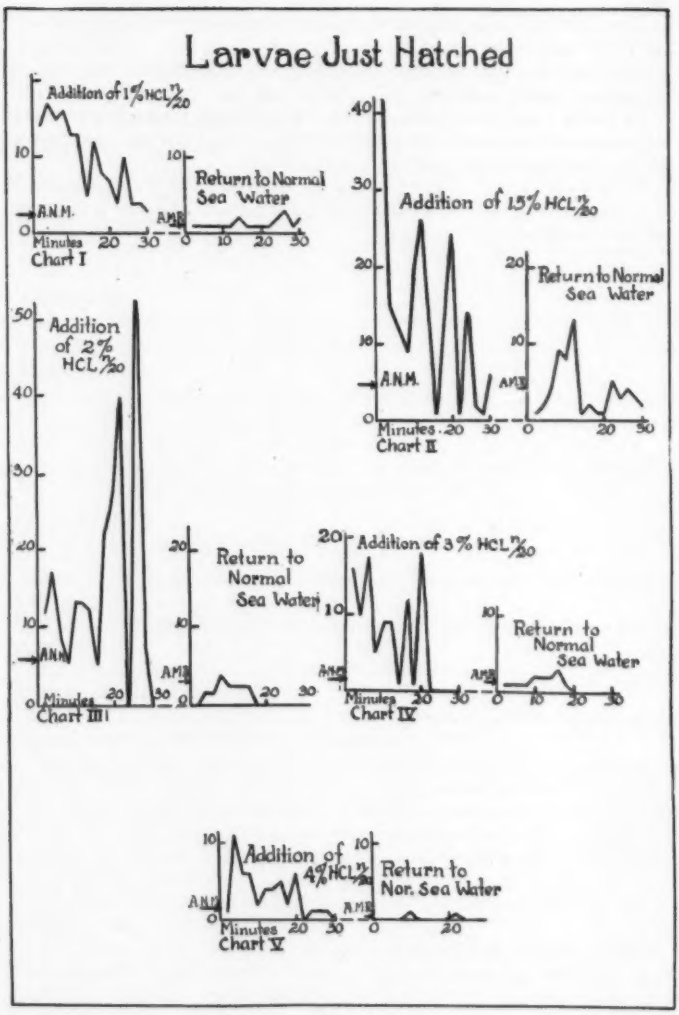
The arrow marked A. R. R. indicates the number of seconds required for 10 respiratory movements when returned to normal sea water from the acidulated water.

Chart XIV. shows the influence of high concentrations of CO_2 on the respiratory rate in two individuals (*A* and *B*).

Chart XV. shows the fall of the respiratory rate which is observed at the end of each spontaneous movement.

On Tables I. and II. the average number of movements (that is, the number of separate movements which took place regardless of extent and character) is given for each 10-minute period under different conditions.

In Tables III. and IV. is given the average numerical value (that is, the amount of movement, using the coil to one side for unity) for each 10-minute period under different conditions.

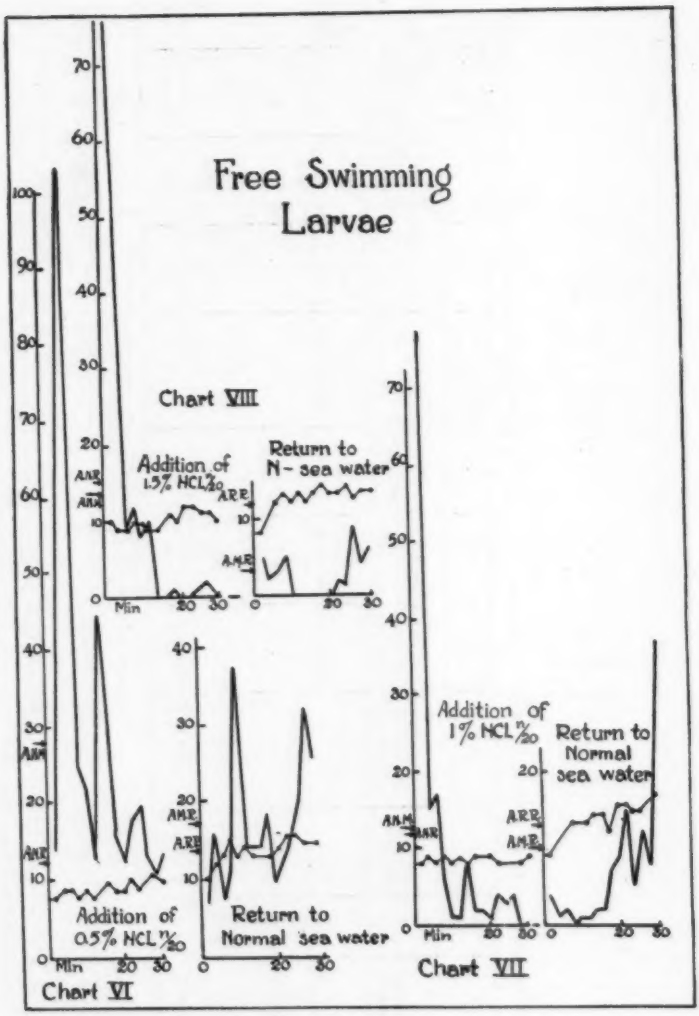

Larvae Just Hatched
Addition of 1% HCL 1/20
A.N.M.
Minutes
Chart I
Return to Normal Sea Water
Addition of 15% HCL 1/20
Return to Normal Sea Water
Chart II
Addition of 2% HCL 1/20
Chart III
Return to Normal Sea Water
Addition of 3% HCL 1/20
Return to Normal Sea Water
Chart IV
Addition of 4% HCL 1/20
Return to Nor. Sea Water
Chart V

CHARTS I–V.

TABLE I.

NUMBER OF MOVEMENTS IN 10 MINUTE PERIODS OF NEWLY HATCHED LARVÆ UNDER DIFFERENT CONCENTRATIONS OF CO_2.

1	2	3	4	5	6	7	8	9	10	11	12	13
% HCl n/20.	Partial Pressure CO_2.	P_H.	Number of Specimens.	Average Number of Normal Movements in 10 Minutes.	Average Number of Movements Each 10 Minutes under Increased CO_2.				Average Number of Movements per 10 Minutes when Returned to Normal.			
					1st 10 Minutes.	2d 10 Minutes.	3d 10 Minutes.	Number of Specimens not Stimulated.	Number of Specimens.	1st 10 Minutes.	2d 10 Minutes.	Number of Specimens Aberrant.
1	.010	7.0	13	6	9.6	4.7	5.4	4	8	1.4	3	2
1.5	.0185	6.64	4	5.15	10.3	3.5	4.5	1	4	6.3	7.7	2
2	.025	6.42	8	13.2	13.5	6.7	4	3	6	9	17.3	2
3	.038	5.96	9	11.4	11.4	7.5	0.7	4	9	10.2	7.6	2
4	.052	5.00	4	3.4	9.5	1.5	1.5	0	4	0.25	0	1
Averages and totals.....			38	8.4	10.9	5.3	3.5	12	31	5.4	7.3	9

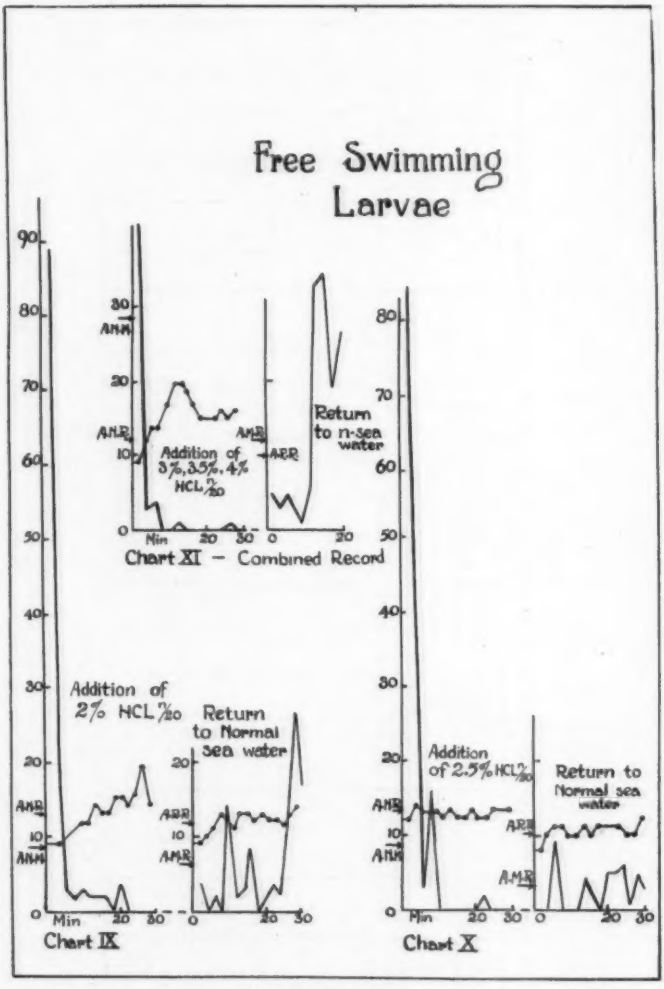


CHARTS VI–VIII.

TABLE II.

NUMBER OF MOVEMENTS IN 10 MINUTE PERIODS OF FREE SWIMMING LARVÆ UNDER DIFFERENT CONCENTRATIONS OF CO_2.

1	2	3	4	5	6	7	8	9	10	11	12	13
% HCl n/20.	Partial Pressure CO_2.	P_H.	Number of Specimens.	Average Number of Normal Movements in 10 Minutes.	Average Movements of Each 10 Minutes with Increased CO_2.			Number of Specimens not Stimulated.	Average Movements per 10 Minutes when Returned to Normal.			Numoer of Specimens Aberrant.
					1st 10 Minutes.	2d 10 Minutes.	3d 10 Minutes.		1st 10 Minutes.	2d 10 Minutes.	3d 10 Minutes.	
0.5	.005	7.5	3	21	46	17.7	22.3	0	15	15	26.5	0
1.0	.010	7.0	3	11.1	15.3	3.3	2.5	2	3.3	11	25.7	0
1.5	.0185	6.64	3	14.3	24.3	3.3	1.7	1	3.3	0.3	9.3	0
2.0	.025	6.42	4	14.2	8.2	4.5	1.8	2	4.0	2.5	12	0
2.5	.0315	6.2	2	10.3	10.0	0	0	1	3	3	6.5	
3.0	.038	5.96	1	20	6	0	0	1	15	22	—	0
3.5	.045	5.60	1	1.3	6	0	0	0	2	1	22	1
4.0	.052	5.00	2	27.5	2.5	0	0.5	2	25	3	10	0
Averages and totals.....			19	15.4	17.2	4.6	5.2	9	8.1	6.5	16	1

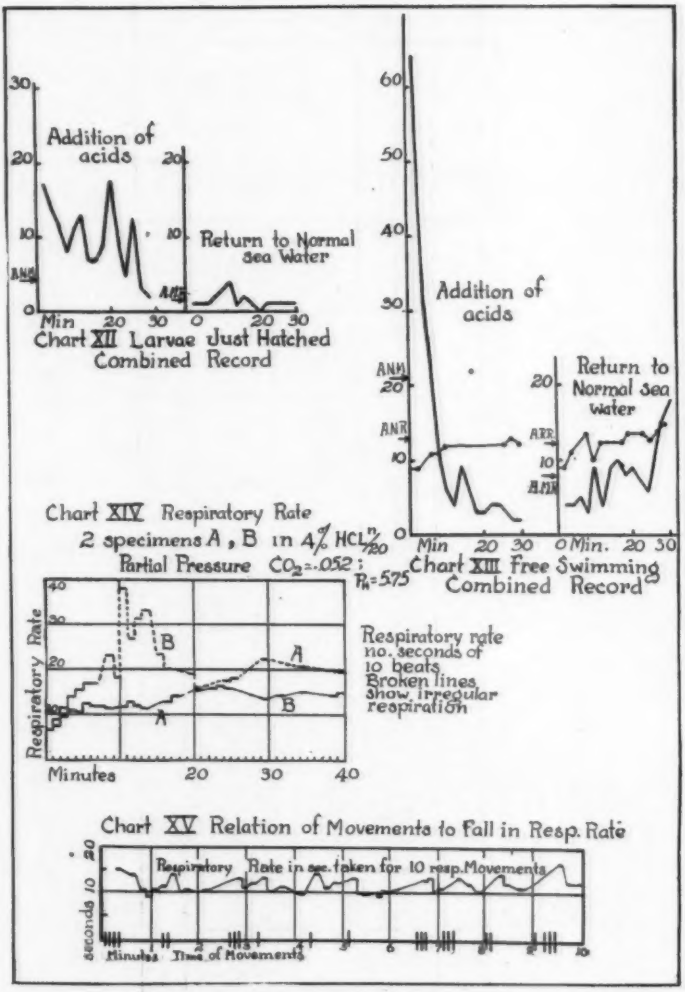

Free Swimming Larvae
Addition of 3%, 3.5%, 4% HCL/20
Return to n-sea water
Chart XI – Combined Record
Addition of 2% HCL/20
Return to Normal sea water
Chart IX
Addition of 2.5% HCL/20
Return to Normal sea water
Chart X
Min

CHARTS IX–XI.

TABLE III.

NUMERICAL VALUE IN 10 MINUTE PERIODS OF NEWLY HATCHED LARVÆ UNDER DIFFERENT CONCENTRATIONS OF CO_2.

1	2	3	4	5	6	7	8	9	10	11	12	13	14
% HCl n/20.	Partial Pressure CO_2.	P_H.	Number of Specimens.	Average Number of Normal Movements in 10 Minutes.	Average Numerical Value of Each 10 Minutes under Increased CO_2.				Average Numerical Value per 10 Minutes when Returned to Normal.				Number of Specimens Aberrant.
					1st 10 Minutes.	2d 10 Minutes.	3d 10 Minutes.	No. Specimens not Stimulated.	Number of Specimens.	1st 10 Minutes.	2d 10 Minutes.	3d 10 Minutes.	
1	.010	7.0	13	41.6	154.7	103.2	51.2	2	8	5.9	6.5	18.7	2
1.5	.0185	6.64	4	50.2	193	148	48.2	1	4	48.5	49.5	51.2	1
2	.025	6.42	8	68.4	112.4	53	—	4	6	22	15.1	—	0
3	.038	5.96	9	24.7	113.1	39.4	—	1	9	11.1	22.5	23.5	0
4	.052	5.00	4	24.5	42.2	42.2	6.7	1	4	0.3	0.0	0.8	1
Averages and totals.....			38	42.3	128.1	75.8	42.1	9	31	15.3	17.5	22.8	4


Addition of acids
Return to Normal Sea Water
Min 20 30 0 20 30
Chart XII Larvae Just Hatched Combined Record
Addition of acids
Return to Normal Sea water
Min 20 30 0 Min. 20 30
Chart XIII Free Swimming Combined Record
Chart XIV Respiratory Rate
2 specimens A, B in 4% HCl n/20
Partial Pressure CO_2=.052;
P_H=575
Respiratory Rate
Minutes 20 30 40
Respiratory rate no. seconds of 10 beats. Broken lines show irregular respiration
Chart XV Relation of Movements to Fall in Resp. Rate
Respiratory Rate in sec. taken for 10 resp. Movements
seconds
Minutes
Time of Movements

CHARTS XII–XV.

TABLE IV.

NUMERICAL VALUE IN 10 MINUTE PERIODS OF FREE SWIMMING LARVÆ UNDER DIFFERENT CONCENTRATIONS OF CO_2.

1	2	3	4	5	6	7	8	9	10	11	12	13
% of HCl n/20.	Partial Pressure CO_2.	P_H.	Number of Specimens.	Average Number of Normal Movements in 10 Minutes.	Average Numerical Value of Each 10 Minutes under Increased CO_2.				Average Numerical Value Each 10 Minutes when Returned to Normal.			Number of Specimens Aberrant.
					1st 10 Minutes.	2d 10 Minutes.	3d 10 Minutes.	Number of Specimens not Stimulated.	1st 10 Minutes.	2d 10 Minutes.	3d 10 Minutes.	
0.5	.005	7.5	3	287.4	497	219.6	195.3	1	162.3	130.6	156	
1.0	.010	7.0	3	247	249	21	27		21	47	168.6	1
1.5	.018	6.64	3	137.3	337	4.6	6.3	1	25.3	0.6	63.6	
2.0	.025	6.42	3	157.8	196.2	16.2	2.7	2	48	20.7	125.3	
2.5	.0315	6.2	3	93	267.3	3.3	0.3	1	20	23.3	53.5	
3.0	.038	5.96	1	245	43	0.0	0.0	1	107	18.8	0.0	
3.5	.045	5.60	1	8	60	0.0	0.0		20	20.3	0.0	1
4.0	.052	5.00	2	503	117.5	0.0	2.0	1	18	0.0	0.0	
Averages and totals.....			19	211.9	261.9	41.7	36.2	7	52.2	37.1	89.5	2

THE RELATION OF BODY TO ENVIRONMENTAL TEMPERATURES IN TURTLES, *CHRYSEMYS MARGINATA* BELLI (GRAY) AND *CHELYDRA SERPENTINA* (LINN.).

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Although a considerable number of workers have recorded their observations on the relation of body temperature to that of the environment of different so-called cold-blooded animals, only a comparatively few of these refer especially to the reptiles, as a group, or to the turtles in particular. With but the exceptions indicated below, all the records from the early workers were based upon a limited number of individuals taken for the most part at random within narrow limits of normal but slight environmental changes. It has thus become quite generally assumed by biologists that turtles together with other cold-blooded animals approximate the temperature of their surroundings. In view of the fact that turtles as a representative group of the reptiles have an unique phylogenetic position, spanning the gap as it were between the warm-blooded birds on the one hand, and the cold-blooded amphibians on the other, it was thought that a study of their body-temperature changes when followed through high and low critical temperatures as well as the ordinary non-critical ranges, might yield interesting data.

The earliest observation of the body temperature in turtles is probably recorded by Walbaum (1), in 1782. He found the temperatures differed only one or two degrees from that of its environment and fluctuated with it. Following Walbaum a considerable number of workers made similar observations during the first half of the nineteenth century. Milne-Edwards (2) in 1863, after giving a critical review of the work done on these forms by Czermach (3), Murray (4), Tiedemann (5), Davy, J. (6), and Valenciennes (7), concluded that the body temperatures

varied but little from that of the environment wherein the limits did not exceed 4 degrees C. It is of interest that the same author noted the observations of Valenciennes (7) which were later substantiated by Sclater (8), that the female python when coiled about the eggs during incubation, maintained a body temperature considerably above that of the surrounding air. Sclater found on comparing the body temperature of the incubating female with that of the male, that during the height of incubation the difference was as much as 10 degrees C., and that she was some 20 degrees C. above the surrounding air. Whether this marked increased in heat production was brought about by bringing into play a thermal regulatory mechanism, or was due to other causes was not stated and has not yet been learned. Similar observations were reported in 1881 by Forbes (9), with somewhat less conspicuous differences. The greatest difference of the air and the surface coils of the snake was about 6 degrees in the male, and about 9 degrees C., in the female. It was noted in this study that the female took no food and was comparatively inactive for weeks before and during incubation.

In 1903, Martin (10) working with the respiratory exchanges in Monotremes and Marsupials included some observations on the blue-tongue lizards (*Cyclodus gigas*). These, five in number, he carried through changes in temperatures varying from 5 to 39 degrees C., within a calorimeter. At room temperature they were comparatively active but became quite inactive at 5 degrees. On warming they increased in activity up to until about 30 degrees and above this their activity diminished. The body fluctuations accompanying these changes were noted. Throughout the middle ranges (10–35 degrees C.) the body temperature was a function of the environment but the CO_2 production was fairly constant. At the extremes (below 10 and above 35) as shown in his plots, Fig. 3, sharp breaks occur, with approximation to Van't Hoff's law. Notwithstanding that he kept the animals in an environment of between 39 and 40 degrees C., for over two hours he was unable to get their mean temperature above 38.5. In their work on certain of the cold-blooded animals Rogers and Lewis (10) followed the body temperature fluctuations in only two representatives of the vertebrates, the fishes (goldfish) and the amphibians (sala-

manders) but concluded that neither of these forms possessed mechanisms for either heat production or heat loss.

Experimental.—Turtles were collected in the vicinity of Lakeside Laboratory, Lake Okoboji, Iowa. The painted variety was unusually abundant in July and August in the shallow cove at Miller's Bay. The snappers were comparatively rare and could not be obtained in considerable numbers. Most of these were

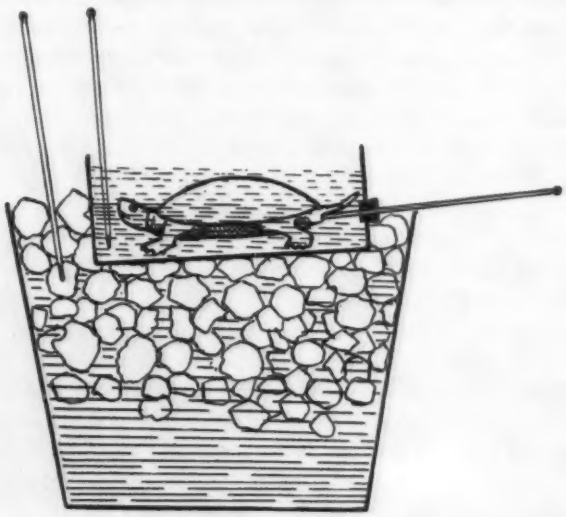

FIG. 1. Diagram of relationships of the apparatus used in cooling experiments. The animals were made fast by clamp in the smaller container surrounded by water, and this subjected to an ice bath. Temperatures were read from the three thermometers shown.

taken from Hottes' and Marble Lakes, west of Spirit Lake, while others were found along the shores of Little Sioux river and in the neighborhood of Hanging Bog. Suitable specimens of various sizes were placed in a live box as caught, and from there were taken into the laboratory as needed.

Individuals were weighed and mounted in such a way that their temperature changes might be recorded. Those placed in air as a surrounding medium were fixed to a board by heavy rubber bands slipped about the carapace and plastron and spaced so that the animal was unable to release itself by kicking.

The board was then supported at either end high enough above the table that the animal could not get sufficient traction to crawl. In this position the tail could be tied back and the thermometer inserted in the rectum.

Experiments with water as the surrounding medium were more difficult to carry out, and involved the construction of special containers which could be adapted to different sizes of individuals (Fig. 1). Shallow tin dishes of convenient sizes were found to fit the purpose, allowance being made so that a stoppered thermometer could be supported at the side. In mounting, the thermometer could be slipped into the rectum, and the animal made fast by adjustable wire clamps. Mounted in this way the specimen could be subjected to an ice mixture and its changes in body temperature as well as those of the environment be recorded.

Although some slight variations in the experimental technique were followed for checking purposes, in general the procedure in all cases involved the tabulating of the date of the experiment and the time intervals of the observations, the room, as well as the immediate environmental and the subject's temperature, the subject's number and its weight, and remarks on the behavior and stages of activities of the animal, under the conditions of the experiment. Some forty experiments exclusive of several preliminary observations, furnish data for this report, these being apportioned about equally between the two forms of turtles studied. For convenience, the discussion of results are grouped under the following captions:

Body Temperature Fluctuations in the Non-critical Ranges.— In a series of thirteen experiments, the diurnal and nocturnal fluctuations in body temperature were taken on individuals of the painted and snapping varieties, both in air and water. A typical chart of observations taken at intervals over a twelve-hour period from a painted turtle mounted in air, weighing 485 grams is given in Table I.

In general it is noted that the body temperature lags from one to three degrees as the temperature rises, and remains slightly above as it is lowered, being slightly effected by the state of the activity of the animal. The same fluctuations are apparent during the nocturnal intervals. Similar observations were made

upon the snapping turtles in air and in water but since comparable results were obtained through these ranges it is unnecessary to cite typical protocols.

TABLE I.

AUGUST 1, 1924. OBSERVATIONS SHOWING THE TYPICAL FLUCTUATIONS OF BODY TEMPERATURES WITH ENVIRONMENTAL TEMPERATURES IN THE PAINTED TURTLE.

Date.	Time.	Room Temp.		Subj. Temp.		Diff.		Activity.
Aug. 1	8.00 A.M.	63° F.	17.5° C.	61° F.	16.0° C.	−2.0° F.	−1.0° C.	Quiet
	9.00	63	17.5	61.5	16.3	1.5	−0.8	"
	9.30	63	17.5	63	17.5	0	0	Active
	10.00	64	18	64	18	0	0	"
	10.30	65	18.5	64	18	−1.0	−0.5	"
	11.00	67	19.5	65	18.5	−2.0	−1.0	Quiet
	11.30	69	20.5	65	18.5	−3.0	−2.0	"
	12.00	70	21.0	68	20	−2.0	−1.0	Active
	12.30 P.M.	71	21.5	69	20.5	−2.0	−1.0	"
	1.30	70.5	21.3	70	21.0	0.5	0	"
	2.00	70	21.0	70	21.0	0	0	"
	3.00	69	20.5	69.5	20.5	0.5	0	"
	4.00	67	19.5	69	20.5	2.0	1	"
	7.00	67	19.5	69	20.5	2.0	1	"
	8.00	66.5	19.5	68	20.0	1.5	0.5	Quiet

Body Temperature Fluctuations on Cooling.—In these experiments *twelve* specimens of the painted variety ranging in weights from 265 to 712 grams, and ten snappers ranging from 152 to 1725 grams were used. Some of the representatives of each group were subjected to rapid environmental drops while in others the drop was made more slowly. This procedure was followed because in the preliminary observations it was noted that differences in rate of cooling seemed to effect slightly the body temperature fluctuations. In some cases the same individuals were rechecked on different days following the same procedure, and while slight divergences appeared in the records, the same general tendencies were apparent. Illustrating the chief points concerned with the rapid cooling of the painted variety data are given in Table II., which is taken from an experiment performed on Aug. 1, on a medium-sized (360 gms.) turtle, and which is fairly typical of them all.

This particular experiment began at nine o'clock in the morning and extended over five hours, the rapid drop in environmental temperature occurring during the first half hour. Although the

TABLE II.

PAINTED TURTLE, ENVIRONMENTAL AND BODY TEMPERATURES. RAPID COOLING.

Time.	Room Temp.		Sub. Temp.		Env. Temp. H_2O.		Diff.		Activity.
	F.	C.	F.	C.	F.	C.	F.	C.	
9.00 A.M.	63°	17.5°	63°	17.5°	60°	15.5°	3°	2°	Quite active
9.30	63	17.5	62	16.8	36	3.5	26	13.3	Slow move
10.00	64	18	62	16.8	35	3	25	13	" "
10.30	65	18.5	61.5	16.3	34.5	2.5	27	12.8	" "
11.00	67	19.5	61	16	34	2	27	14.0	Quiet
11.30	69	20.5	58	14.5	34	2	24	12.5	"
12.00	70	21.0	56.6	13.3	33	0.5	23	12.5	"
12.30 P.M.	71	21.5	56.5	13.2	33.5	0.6	23	12.3	"
1.30	70.5	21.3	56	13	33	0.5	23	12.5	"
2.00	70	21	56	13	34.5	2	21.5	12	"

active movements become somewhat subdued when the animal is subjected to a rapid drop in environmental temperature during the first half hour, as indicated in the table, the fact that the body temperature remains at so conspicuously a high level would seem to indicate that the shock of the cold acts as a stimulus and is compensated for, perhaps nervously, by a sudden

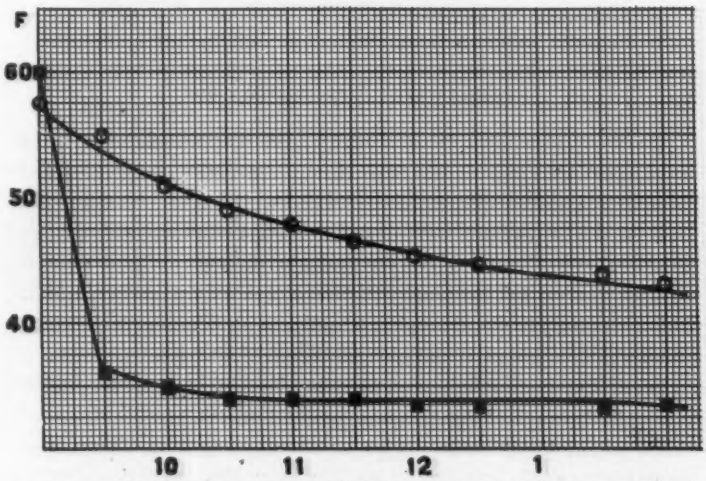

FIG. 2. Plot showing the average body temperature drop from seven painted turtles, correlated with a rapid environmental drop. The body temperatures are represented by circles, the environmental temperatures by squares.

increase in the production of heat. The temperature correlations from seven similar experiments are averaged and plotted against the time intervals and are shown in Fig. 2. Although the body temperature as a rule shows an immediate drop, it is not nearly commensurate with the rapid decline of the surrounding water, and it is conspicuous that during the succeeding four hours the differences are considerable and on the average remain from 8 to 10° F. above that of the environment.

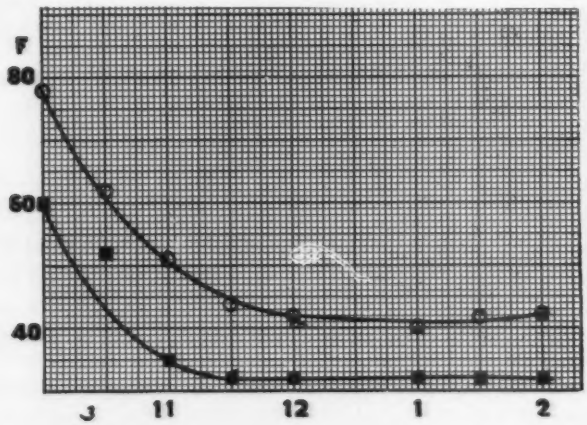

FIG. 3. Plot showing the average body temperature drop from five painted turtles, correlated with a slow environmental drop.

On the following and succeeding days, experiments with the same and other individuals of about equal weight were performed where the animals were subjected to slow cooling which extended over a period of two hours and were then maintained at the temperature of melting ice for another interval. Data from a typical experiment on a painted turtle, are shown in Table III., and the plot of the average fluctuations in temperatures from five records is given in the plot of Fig. 3.

From the table it is noted that at first the animals are quite active but this activity gradually merges into a period of quiescence as the temperature drops to about 45 degrees where, due probably to slight increase in activity, the drop is checked.

In the case of the body temperature changes in the snapping turtles less conspicuous differences are noted. They follow more

TABLE III.

PAINTED TURTLE, ENVIRONMENTAL AND BODY TEMPERATURES; SLOW COOLING.

Time.	Room Temp.		Subj. Temp.		Env. Temp.		Diff.		Activity.
Aug. 2									
8.30	67° F.	19.5° C.	65.5° F.	18.5° C.	62° F.	16.5° C.	3.5° F.	2° C.	Active
9.00	72	22.2	60	15.5	52	11	8	4.5	"
9.30	75	24	55	12.5	47	8.5	8	4	Quiet
10.00	77.5	25.4	54	12	44	6.9	10	6	Slight activity
10.30	80.3	27	49	9.5	43	6	6	3	Quiet
11.00	81	27.5	45	7	36	3	9	4	"
11.30	82	27.9	43	6	33	0.5	10	5.5	Slight activity
12.00	83	28	42	5.5	33	0.5	9	5	Active
12.30	83	28	42	5.5	33	0.5	9	5	"
1.30	83	28	42	5.5	33	0.5	9	5	"

closely the external environmental drop than the painted form when cooled by either procedure. The chief point of interest in the comparisons is the location of the point of check in the drop which is five degrees lower on the average for the snapping turtle. No doubt these differences may be correlated to some extent, with the comparative differences in extent of soft body parts as compared to the harder parts in the two forms, but data on these points are not yet available. A typical protocol of the experiments with the snapping turtles is as follows. Four snappers were placed in containers at 10 o'clock on August 5th, in water drawn from the tap at a temperature of 60 degrees. They were subjected to slow cooling by the addition of ice to the outer container and records taken at intervals for four hours. In Table IV. are given data from specimen *C* of the series, weighing 582 grams and which is typical of them all.

TABLE IV.

SNAPPING TURTLE. ENVIRONMENTAL AND BODY TEMPERATURES.

Time.	Room Temp.		Subj. Temp.		Env. Temp.		Diff.		Activity.
10.00 A.M.	81° F.	27° C.	78° F.	25.7° C.	60° F.	15.5° C.	18° F.	10.2° C.	Active
10.30	82	27.9	62	16.5	52	11	10	5.5	"
11.00	84	29	51	10.5	35	1.5	16	9	Quiet
11.30	85	29.5	44	6.7	32	0.0	12	6.9	"
12.00	86	30.2	42	5.5	32	0.0	10	5.5	Active
1.00	86	30.2	40	4.4	32	0.0	8	4.4	"
1.30	86	30.2	42	5.5	32	0.0	10	5.5	"
2.00	86	30.2	42	5.5	32	0.0	10	5.0	"

A similar series of experiments was performed on four specimens (*C*, *B*, *N* and *O*) on the following day and the average figures of these contribute to the plot, Fig. 4. The body tem-

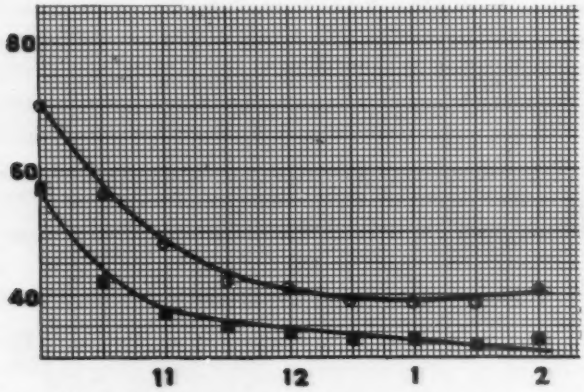

FIG. 4. Plot showing the average body temperature drop from four snapping turtles correlated with a slow environmental drop.

perature curve shows a comparable drop with that of the environment during the first hour and a half but is checked at about 40 degrees.

Body Temperature Fluctuations on Warming.—Two procedures were used in these experiments. On warm days with bright sunlight the animals were placed in containers either in air or in slight amount of water and put directly into the warm rays of the sun. In other cases the temperature of the containers was slowly raised by use of an alcohol lamp through a copper conducting unit. The results seemed not to differ greatly in either procedure. As a rule it was found that the animals could withstand gradual increase in temperature in air better than in water. This was due in part, no doubt, to slight though appreciable transpiration afforded in the former condition. As illustrative of the reactions of the painted variety to gradual increase in temperature, data from specimen *I* are given in Table V. This particular experiment together with others was carried out on an unusually favorable day, August 6. It was noted that hundreds of turtles were basking in the afternoon sun on old logs in the cove north of the laboratory, and it was thought

that observations under experimental conditions might yield interesting although not exactly comparable results. The specimens were taken directly from the lake and fastened in containers in direct sunlight, one thermometer giving body readings and another, in air, the environmental changes. The surface temperature of the lake at this time was 20.5 degrees C. or 69 degrees F., and the initial temperatures of the turtle as they were taken, was only a few degrees less. But at the start of the experiment, after a lapse of some fifteen minutes, due to excitement and activity in the containers, it had approximated the air temperature.

TABLE V.

Time.	Subj. Temp.		Env. Temp.		Diff.		Activity.
Aug. 6							
1.45 P.M.	77° F.	25° C.	76° F.	24.5° C.	1° F.	0.5° C.	Very active
2.00	78	25.7	79	26	1	0.3	" "
2.15	79	26	80	26.8	− 1	−0.8	" "
2.30	92	33.5	106	41.2	−14	−7.7	Gasping, frothing, moisture about eyes. Active.
2.45	102	39	111	44.5	− 9	−5.5	Gasping, frothing, small droplets about eyes.
3.00	99	37.3	109	43	−10	−6.3	Rapid breathing.
3.15	93	34	104	40.1	−11	−6.1	Active, gasping, rapid breathing.
3.30	90.1	32.5	95	35	− 5	−2.3	Restless but frothing
4.00	80	26.8	80	26.8	0	0	Slowly moving

Whether or not the same relative increase in body temperature occurs in the case of the animals observed on the log, is of course, questionable. It was noted that the appendages were outstretched as well as the neck and that after an interval of an hour or more thus exposed, as a rule, a return to the water was made. No doubt also, some slight air currents played upon the body surface, thereby favoring any transpiration that might have occurred. Under experimental conditions, the operation of such factors to help keep the temperature down was very limited.

The average fluctuations in body temperature of five individuals are plotted against increased environmental temperature and shown in Fig. 5. It is noted there are points on the plot of the environmental curve which are somewhat widely displaced.

This is due to the fact that it is difficult to control the environmental temperature throughout the whole period, although in general, the trend of increase and decrease is fairly definite.

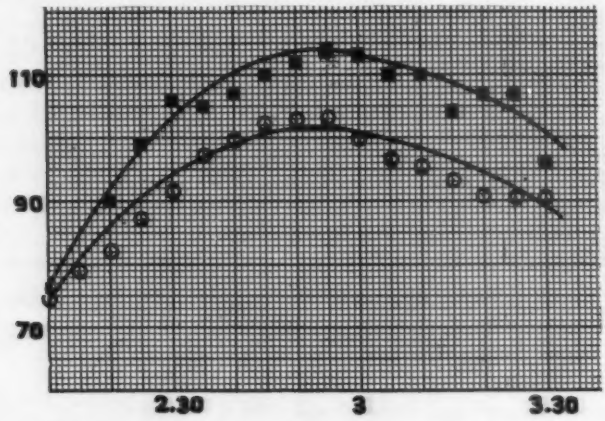

FIG. 5. Plot showing the average body temperature fluctuations of five painted turtles correlated with environmental rise.

The snapping turtle, in experiments under similar conditions, seems to indicate the same general trend, although these animals apparently do not increase in body temperature as rapidly, especially during the initial rise of environmental temperature as does the painted form. This slight difference, however, is transient if the increase in environmental temperature is steady, and eventually at the high critical points the body temperature as well as the reactions in this form simulate those in the other form. Data averaged and plotted from four individuals and checked against the environmental rise are shown in Fig. 6. It is noted on comparison with the preceding plot that increments of environmental temperature are not quite as effective in raising the body temperature in the snappers as in the painted variety. On anatomical grounds one is tempted to attribute these differences in part to a greater radiating surface of soft parts exposed in the snappers, for it is well known that the relative extent of the plastron in this form is considerably less than it is in the painted form. Whether there are in addition, physiological differences in the two forms can not at this time

be stated on the basis of these experiments alone. The comparative behavior of the two forms taken as criteria, would certainly suggest that there might be. Thus the typical signs of discomfort, with rapid respiration and frothing about the mouth and the accumulation of moisture around the eyes appear

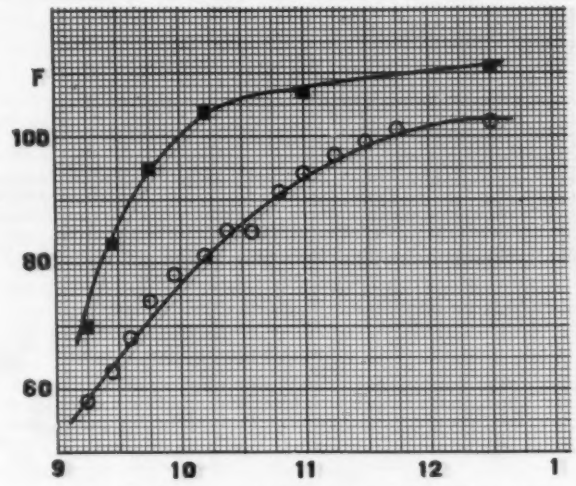

FIG. 6. Plot showing the average body temperature fluctuation of four snapping turtles correlated with environmental rise.

at a body temperature, some ten degrees higher in the snapper than is the case with the painted turtle. At the high critical temperatures however, little differences in endurance could be noted in the two forms. From the limited data accumulated on this point, it appears that neither form withstands a body temperature maintained at between 102 and 105 degrees F. (39–41° C.) for thirty minutes or longer, and in the majority of individuals death results in a much less time.

When placed in water as the surrounding medium and subjected to gradual increase in temperature animals of both groups are apparently incapacitated in their resistance. A typical experiment is cited in Table VI. This animal, a painted turtle, weighing 520 grams, was placed in a container of water at 9.30 on August 2. The water was drawn from the laboratory tap at practically room temperature and was heated during the

extent of the experiment by a small alcohol flame through conduction. It is noted that the body temperature lagged slightly but kept pace with the environmental rise. At the end of two and one half hours, the temperature had reached the critical point and the animal was removed in a moribund condition from which it did not recover. Several other experiments with other individuals eventuated similarly.

TABLE VI.

Time.	Room Temp.		Subj. Temp.		Envir. Temp. (H_2O).		Diff.		Activity.
9.30	75° F.	23° C.	69.5° F.	21° C.	64° F.	18° C.	5.0° F.	+3.0° C.	Active
10.00	77.5	23.3	79	26.5	81	27.3	−3	− .8	Active
10.30	80.3	27	88	31.2	88	31.2	0	0	Very active
11.00	81	27.3	92	33.5	99	37.5	−7	−4	Scratching. Head outstretched above water.
11.30	82	27.9	104	40	108	42.5	−4	−2	Quiet, dead when removed.

Summary.—1. The rectal fluctuations of the common painted and snapping turtles of various sizes and weights are followed through ranges of non-critical and critical high and low environmental changes. In the non-critical range (50–80° F., 10–27° C.) in both forms the fluctuations are found to vary from 3 to 6 degrees F. (1.5 to 3° C.). When the environmental drop is rapid on cooling the rectal reading shows a somewhat greater lag than when cooled more rapidly. In both procedures a check in drop appears at about 40 degrees F., (4.5° C.) and there is maintained for a considerable interval of time.

2. Accompanying these temperature changes are noted differences in physiological activities, with muscular action at the outset which merges into a period of comparative quiet, this in turn followed by slow continuous movements.

3. Increase in environmental temperature is accompanied by a corresponding rise in body temperature and as a rule this is fatal if maintained at 102 to 105 degrees F., for any considerable

time, (30 minutes or more). At 80 degrees and above, animals show marked increased activity; signs of discomfort with rapid respiration; a frothing about the mouth and an accumulation of moisture upon the head and about the eyes.

4. In the absence of concrete data on comparative metabolic rate at different temperatures, these facts are tentatively interpreted to mean that there is in turtles a slight tendency to compensate for critical temperature changes in their environment.

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ON THE COLOR CHANGES IN THE SKIN OF THE LIZARD *PTYCHOZOÖN HOMALOCEPHALUM*.

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The javanese lizard *Ptychozoön homalocephalum* Crvdt. can change its color in some degree in connection with the difference in the color of its surroundings. In bright sunlight the dorsal surface of the animal is light gray with the exception of a number of pronounced blackish brown stripes which have a zigzag course in transversal direction and some spots of the same color at the lateral parts of its head and neck. In strongly shady surroundings the color of the skin between the transversal stripes darkens and assumes almost the same dark color as the stripes, which thereby become almost invisible. With these animals, which very commonly occur in 's Lands Plantentuin at Buitenzorg I have made some experiments to make out whether the change of color is controlled by the vision of the animals or if it is only due to the influence of light acting on the skin directly.

The experiments were made in August 1921 in the Treub-laboratorium at Buitenzorg with animals captured in the Plantentuin, in most cases by natives. I kept the animals in glass vessels with a diameter of about 20 cm., the bottom and sides of which were covered by white paper or black velvet. In these vessels they behaved quite calmly. The experiments consisted in the comparison of the color of the skin of the same animal (1) in a white-covered vessel, (2) in a black-covered vessel, and (3) in a white-covered vessel whilst its eyes were covered with a not transparent cap. The latter consisted of the cut-off digit of a rubber glove, in the top of which a small hole was cut. This cap was pushed over the head of the animal. It covered its eyes and the greater part of its head and neck, whilst the end of the snout was free and the respiration was not hindered. When the animal had been in the same conditions for some time (at least a quarter of an hour) I noted the color

of the skin of its dorsal surface and then placed it in another vessel under different conditions.

Two of my animals I have photographed under the influences of the above-mentioned different conditions. From each of these six photographs were made on one morning, in the case of one of them in the following order: (1) head free, on white back-

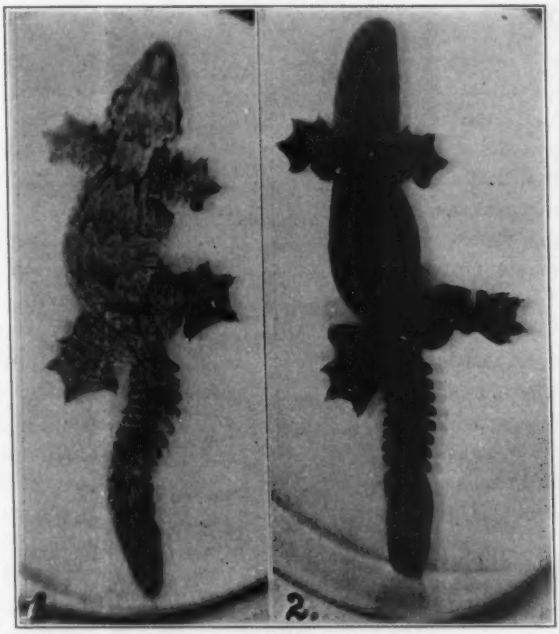

FIG. 1. *Ptychozoön homalocephalum*, on a white background. About $\frac{3}{4}$ nat. size.
FIG. 2. *Ptychozoön homalocephalum*, the animal of Fig. 1, on a white background with covered head. About $\frac{3}{4}$ nat. size.

ground, (2) head covered with cap, on white background, (3) head free, on black background, (4) head covered with cap, on white background, (5) head free, on black background, (6) head free, on white background. In a second series of photographs, taken from the other lizard, the succession of experiments was different, but with each exposure the conditions were different from those before and twice the animal was photographed under the same conditions. The photographs taken from animals in

the same conditions always gave the same results of the coloration of the skin.

As is shown by Fig. 1 the transversal stripes are very clearly visible when the animal with uncovered head is on a white background. The parts of the skin between these stripes have a light gray color which is brightest immediately behind the stripes

FIG. 3. *Ptychozoön homalocephalum*, another specimen than that of Figs. 1 and 2, on a black background. About $\frac{2}{3}$ nat. size.

and somewhat darker before them. A sharp contrast with this figure is that of Fig. 2. Here the animal is photographed on a white background, but with covered head. Now the color of the skin is almost uniformly dark gray whilst the transversal dark stripes are more indistinct. Almost the same color is assumed by *Ptychozoön* when it is surrounded by black (Fig. 3). Then also the stripes are only faint and the whole surface is almost uniformly dark gray.

I have made no experiments as to the length of time these lizards require for a complete change of color. Anyhow it takes less than a quarter of an hour. The photographs have been made with Ilford Panchromatic Plates, which were each exposed during one and a half minutes. The animals remained during this comparatively long time almost immobile on the same spot. The plates are developed in the same liquid during quite the same time. They have not at all been retouched or altered in any way. The positives are made with gaslight paper, those of Figs. 1 and 2 are exposed for quite the same time and have afterwards been worked out into a cliché together. Therefore these figures give reliable data for a comparison of the color of the skin of the same animal on a white background with free and with covered eyes. The technical peculiarities given above are mentioned to prove that the two figures give accurate data for the differences of the color under these different circumstances. Fig. 3 can only less directly be compared with the others, but it shows clearly enough that on a black background the color of the skin is of an almost even shade. The plate of this figure has been exposed for the same time as those of Figs. 1 and 2, but owing to the black background it is more or less underexposed. Therefore the animal appears brighter than it was in reality.

The external influences in these experiments were almost quite constant. I studied the color changes of *Ptychozoön* always on quite the same spot in diffuse daylight at a distance of circa 1 m. from the window. The *intensity of the light* was always practically the same, the time being the dry monsoon, when between 8 and 12 A.M. the intensity of the light in the tropics is quite equal. The difference in maximum and minimum of the *temperature* (if there was any difference at all) was very slight and also the *degree of humidity* did not vary noticeably during the experiments. The *structure of the substratum* was in all experiments the same. This, however, proved to be of little importance as was shown by a little minor experiment. Once I tried the same experiments by bringing the animals successively on white paper and on black velvet, but the color changes were the same as in the experiments with the animals in differently clad glass-vessels.

From the above stated peculiarities we may conclude that

Ptychozoön, when the external conditions are quite the same except the color of its surroundings, assumes the color which simulates in the highest degree that which it sees with its eyes. In other reptiles no instances are known that the changes of color are influenced by the vision of the animals, and also experiments on this question only gave negative results (cp. van Rynberk, 1906; Fuchs, 1914).

An important fact is moreover that the color of the skin of *Ptychozoön*, when the temperature is constant, becomes lighter on a white background and darkens on a black one. After Parker (1906) the pigment-cells of all reptiles expand in the light, whilst they contract in the darkness. In those cases, where by other authors different conclusions have been made, these would be due to changes of temperature which had not been taken into account by these authors. In those experiments in which the color changes of reptiles alternately in the shade and in the sunlight were studied, heat reactions may have influenced the movements of the chromatophores, in my experiments, however, the animals remained always on the same spot in diffuse daylight. The temperature was always very uniform and the lizards reacted directly on stimuli of white and black surroundings in the above described manner.

A contradiction to Parker's theory is found in the statements given by Thilenius (1897) for *Varanus*. According to this author *Varanus* assumes a dark color in the shade in 45–50°, whilst in the sunlight at a temperature of less than 30° the color becomes light. Parker has expressed his doubts as to the correctness of these temperatures, which might have been read from an ordinary mercury-bulb thermometer. With a more precise instrument the result would have been a much higher temperature. The difference between the true temperature and the one recorded in this case, however, would then have been more than 20°, and this difference is too large to be put on account of the inaccuracy of the instruments. In the case of *Ptychozoön* the color changes take place in the same way as described by Thilenius for *Varanus* and this proves that the chromatophores of at least some lizards contract in light and expand in dark.

In another way Fuchs (1914) has tried to explain the fact that light in some reptiles causes an expansion and in others a con-

traction of the chromatophores. Firstly he points to the fact that larvæ of amphibians show a different reaction to light in the different stages of development. In *Amblystoma* Babàk (1910) found that the very young larvæ react clearly on stimuli of light, becoming dark in the light and light in the shade. The eyes then have as yet no influence on the color changes. Afterwards, when the larvæ have grown older, the light has a completely other effect on the larvæ: the older larvæ become light in the light and assume a dark color in the dark. This reaction takes place under the influence of the eyes.

According to Fuchs the parietal organ, which in young larvæ is well-developed, has the function to impede the contraction of the chromatophores. Afterwards the eyes obtain an influence on the function of the pigment-cells, the illumination of the retina causes then a contraction of the chromatophores. Stimulation of the eyes therefore causes in older larvæ a reaction opposed to that caused by stimulation of the parietal eye. The older the larvæ, the stronger becomes the influence of the eyes as compared with that of the parietal eye. Consequently these animals become dark on a dark background and light on a light one.

In comparison with these different reactions towards light in young and older larvæ of amphibians Fuchs has put forward the hypothesis that in those reptiles, which show an expansion of pigment in light, the impeding influence of the functioning parietal organ is present. In those reptiles which show a contraction of pigment in light according to Fuchs either the parietal organ has lost its function in the course of phylogeny or ontogeny, or the eyes have acquired, as in older larvæ of amphibians, a regulating influence on the reactions towards light, on account of which the original reaction (expansion) was changed into the opposite (retraction).

In *Ptychozoön* no parietal eye is present. The general shape of the organs in this region of the brain is shown in Fig. 4. The epiphysis consists of a closed pouch which by means of a solid trace of cells is connected to the roof of the diencephalon just behind the commissura habenularis superior. It is directed backwards and covered by a protusion of the roof of the diencephalon, the "Zirbelpolster" of German authors. The well-

developed paraphysis is found immediately before the latter. Almost quite the same arrangement and structure of these organs is found in *Platydactylus muralis* L., an allied species, in which the anatomy and development of the parietal organ is described

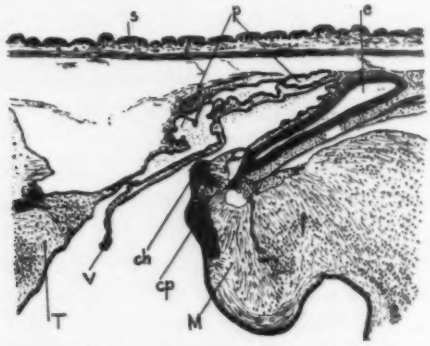

FIG. 4. *Ptychozoön homalocephalum*, newly-hatched specimen. Longitudinal section of a part of the brain. Hæmatoxylin-Delafield, eosin. ×43. *ch*, commissura habenularis superior; *cp*, commissura posterior; *e*, epiphysis cerebri; *M*, mesencephalon; *p*, paraphysis; *S*, skin; *T*, telencephalon; *v*, velum transversum.

at length by Melchers (1900). Besides that from the newly-hatched *Ptychozoön* (with a head-length of 11 mm.) a section of which is shown in the figure, another series of longitudinal sections was made from a younger stage, measuring 9 mm. from snout to occiput. The conditions found here make it highly probable that the development of these organs in *Ptychozoön* is quite the same as that in *Platydactylus muralis*.

In the case of *Ptychozoön* the above-cited hypothesis put forward by Fuchs agrees fairly well with the facts. The data available in the literature, however, are often in contradiction with this hypothesis. In *Platydactylus mauretanicus* the parietal organ is absent and yet this lizard assumes a dark color in the light and becomes light-colored in the dark. On the contrary *Stellio caucasicus* has a well-developed parietal eye and notwithstanding that the animal becomes light-colored in the light and dark in dark surroundings (cp. Studniczka, 1905, and Fuchs, 1914). These two instances already prove that there is insufficient evidence to uphold the above-mentioned hypothesis of Fuchs.

The microscopical structure of the skin in the lighter parts of the dorsal region of *Ptychozoön* differs in some respects with that in the dark stripes. In the light-gray parts (Fig. 5) the epidermis

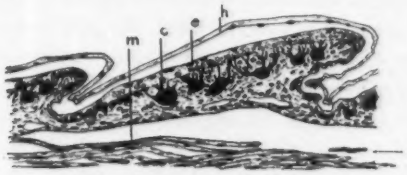

FIG. 5. *Ptychozoön homalocephalum*, newly-hatched specimen. Longitudinal section of a part of a light-colored portion of the dorsal region. Hæmatoxylin-Delafield, eosin. × 100. *c*, cutis with chromatophores; *e*, cell-layer of epidermis; *h*, horn-layer of epidermis, which has loosened from the parts underneath it; *m*, muscles.

contains no pigment at all, the large pigment-cells consist of a large body which is situated in the cutis and a number of ramifications which are directed towards the epidermis and terminate immediately underneath the latter. In the living state a nearly continuous layer of black pigment is distributed under the epidermis by the contraction of the chromatophores. When the pigment-cells expand again, the pigment flows back to the deeper layer of the cutis and the color of the animal becomes much lighter.

In the dark stripes (Fig. 6) the chromatophores of the cutis are usually of a somewhat smaller size than those of the lighter parts of the body, the whole amount of cutis-pigment in a certain

FIG. 6. *Ptychozoön homalocephalum*. Longitudinal section of a part of one of the dark stripes of the dorsal region of the specimen of Fig. 5. Hæmatoxylin-Delafield, eosin. × 100. *c*, cutis with chromatophores; *e*, cell-layer of epidermis, with epidermis-pigment; *h*, horn-layer of epidermis; *m*, muscles.

region, however, is about the same. Besides the cutis-pigment an epidermis-pigment is also present here. The uppermost parts of the cells of the epidermis are crowded with small black pigment granules which are found immediately underneath the

horn-layer of the epidermis. When the chromatophores of the cutis contract, the pigment of the epidermis remains on the same spot and the color of this region retains its black shade. It is only covered by a thin horn-layer and it is therefore much more conspicuous than the pigment of the chromatophores of the cutis, which is covered by the whole ectodermal layer.

The useful effect of these color changes in *Ptychozoön* is a matter of course. The lizards live on the trunk and larger branches of the trees. When they are in shady places they are hidden by their almost uniformly dark color. In the sunlight their light-gray hue equals that of the mossy background. They become still more inconspicuous by the possession of the queer zigzag dark stripes which procures them almost the same color and design as the bark of a tree overgrown with lichens.

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TOXICITY OF OXYGEN FOR PROTOZOA IN VIVO AND IN VITRO: ANIMALS DEFAUNATED WITHOUT INJURY.

L. R. CLEVELAND.[1]

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INTRODUCTION.

The fact that oxygen in an excessive amount is toxic for many if not all forms of life was first demonstrated by the numerous and very thorough experiments of Paul Bert ('74). But the swim bladders of some fishes normally contain oxygen at a pressure of 100 atmospheres (Haldane, '22). The cells lining the bladder apparently are acclimatized to the oxygen. On the other hand, Pütter ('05), working on the respiration of protozoa, states that parasitic forms, such, for instance, as *Opalina*, lived many days in a medium from which practically all the free (gaseous) oxygen had been removed. In view of the work of Pütter and that quoted by Haldane it occurred to me that a study of the toxicity of oxygen for the parasitic protozoa of many animals would be a worthy undertaking.

Wood-eating termites have the most abundant and the most varied entozoan fauna of all animals that have been studied.

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Approximately half the body weight of every worker and nymph, except for a few days after molting, is composed of many kinds of large, intestinal flagellates. Recently molted termites are lacking in pigment and for this reason may easily be distinguished from other individuals. There is no difficulty whatever, then, in being absolutely certain that all animals used in experiments harbor a teeming menagerie of protozoa; consequently termites lend themselves most admirably to many kinds of precise experimentation.

In a previous paper the writer ('25*b*) showed that *Trichomonas termopsidis* was entirely removed from the large Pacific Coast termite *Termopsis nevadensis* Hagen after 24 hours' oxygenation at one atmosphere pressure, and that all the other protozoa (*Trichonympha campanula*, *Leidyopsis sphaerica*, *Streblomastix strix*) were removed after 72 hours' oxygenation. The termites suffered no ill-effects *per se* from the oxygenation, although they died within three to four weeks after their protozoa had been removed. Recently this work has been carried further: four widely separated species of termites from two families have been used; and various intervals and amounts of pressure have been employed.

The bearing which these experiments have on the symbiosis between these termites and their intestinal flagellates will be reserved for a later paper. It is sufficient at present to say that the ability of these termites to live on their normal diet of wood is lost after their protozoa have been removed, regardless of the method employed in removing them.

Many other protozoa-harboring animals, such as cockroaches, earthworms, frogs and rats have been oxygenated. Some of the protozoa of the frog, rat, and man have been grown in cultures which have been oxygenated. And oxygenation experiments have been carried out on several free-living protozoa.

Material.

Of the termite family Rhinotermitidae, *Leucotermes tenuis* Hagen from British Guiana and *Reticulitermes flavipes* Kollar from Maryland were used; in the family Kalotermitidae, *Cryptotermes* sp. from British Guiana and *Termopsis nevadensis* Hagen from Oregon were used. Many thanks are due Dr. Alfred Emerson for *Cryptotermes* and *Leucotermes*.

Cockroaches (*Periplaneta americana?*) were obtained in Baltimore. So were all the parasitic protozoa in culture and most of the free-living protozoa. The exact source of the frogs (*Rana pipiens*), fish, salamanders and rats is not known.

METHODS.

The protozoa-harboring animals and the protozoan cultures were placed in flasks into which tank oxygen of 97 per cent. purity was run until the air was removed, or practically so. The flasks were then clamped down securely and from 1 (760 mm. mercury) to 2.5 standard atmospheres (except for temperature correction) were added to the 1 atmosphere at the time of the experiment. Other details of methods are given under the experiments on the different animals.

EXPERIMENTS.

1. *Termites.*

The effect of oxygenation at various pressures on the protozoa of four genera of termites is given in table I. The minimum time

TABLE I.

TIME REQUIRED AT VARIOUS PRESSURES OF OXYGEN.

Pressure in atmospheres.	To kill all intestinal protozoa of										To Kill *Trichomonas* in Culture from			To Kill Host.		
	Termites.								Cockroach.	Frog.	Frog.	Rat.	Man.	Termopsis.	Cockroach.	Frog.
	Rhinotermitidæ.				Kalotermitidæ.											
	Leucetermes.		Reticulitermes.		Termopsis.		Cryptotermes.									
	Hrs.	Min.	Hrs.	Min.	Hrs.	Min.	Hrs.	Min.	Hrs.	Hrs.	Hrs.	Hrs.	Hrs.	Hrs.	Hrs.	Hrs.
1...	24		*		72		*									
1.5 .	4	30	9		9		7	30								
2...	1	35	4		5		4	30								
2.5 .	1	15	1	40	2		1	55								
3...		50		50	1	5	1									
3.5 .		30		30		40		35	3½	28	6	10	11	45	90	65

* Not killed in ten days.

required to kill the protozoa of termites and other animals was obtained as follows: Several experiments were started at the same time so that any one could be stopped without interfering with

the others, and the experiments were examined one by one until the minimum was found to be within certain limits. Then the experiments were set up again and were examined at various intervals within the known limit. Sometimes it was necessary to repeat the process many times, before a fairly accurate minimum was finally determined. The minimum thus determined was then tested out three times.

Termites were usually examined immediately after having been removed from oxygen and non-motility was the criterion for determining whether or not their protozoa had been killed. It was found that if a few protozoa were motile at the end of the oxygenation period they did not die later. Some hosts which had been freed of motile flagellates were examined at intervals up to ten days, and no protozoa ever appeared in any of them. It is of interest to note that the protozoa disappeared from the intestine very soon—usually within two to four hours—after they had been killed. They were probably digested by the termites.

At one atmosphere the protozoa of *Cryptotermes* and *Reticulitermes* were not all killed in all hosts in ten days; however, they were all killed in a few hosts even in three days. They were all killed in a great majority of hosts in ten days, but in a small number—perhaps about 5 per cent.—some protozoa were alive at the end of ten days.

The protozoa of *Leucotermes* were killed very much more quickly than those of the other termites until a pressure of 2.5 atmospheres was reached. These differences in oxygen toxicity are not correlated with size of termite hosts, for *Termopsis* is approximately twenty times as large as *Leucotermes* and ten times as large as *Cryptotermes*, but *Reticulitermes* and *Leucotermes* are about the same size. Difference in habit may be a factor, but *Reticulitermes* and *Leucotermes* are very similar in habit as well as in structure. The protozoa of these four termites, although all flagellates, are nevertheless quite distinct morphologically, many of them belonging to separate families. Hence, it is possible that the differences in oxygen toxicity may be found to be in the protozoa themselves.

In *Termopsis*, as was true in previous experiments (Cleveland, '25*b*), *Trichomonas* was killed first and *Trichonympha* last until a pressure of three atmospheres was reached. Then a peculiarly

interesting result occurred, *Streblomastix* was killed before *Trichomonas* in many hosts. At a pressure of 2.5 atmospheres, *Trichomonas* and *Streblomastix* are both killed at or about the same time. Regardless of the amount of pressure, the largest protozoa of *Termopsis*, with the exception of one very small form (*Tritrypanoplasma*) not present in all hosts and abundant in only a few, are invariably the last ones to be killed, but in *Cryptotermes* and *Leucotermes* this is not always the case. In *Cryptotermes*, for instance, sometimes all individuals of the largest genus (*Diplonympha?*) are killed when many *Decescovina* are left alive.

Fifty individuals of *Termopsis* were confined in oxygen at 3.5 atmospheres. At the end of forty hours, every individual had become immobile. The experiment was stopped after forty-five hours, when it appeared that every individual was dead, although a few hours later two individuals became feebly motile, and were as active as ever two days later. Forty-five hours at 3.5 atmospheres must be about the time required to kill this termite, which is 67.5 times as long as it takes to kill its protozoa. In other words, oxygen at this pressure is 67.5 times as toxic for the protozoa of termites as for the termites themselves.

The time required to kill the protozoa of these termites at 3.5 atmospheres of oxygen certainly does not injure the termites in the least. Oxygenation at this pressure is surely a very rapid means of freeing termites of their protozoa. It is very much more satisfactory in many ways than incubation (Cleveland, '24), and furnishes a very ready means of determining whether or not all protozoa-harboring termites are dependent on their protozoa to digest their food for them.

In order to determine what effect, if any, partial pressures of other gases, particularly nitrogen, had on oxygen toxicity, termites of each of the four genera that were oxygenated were confined in five atmospheres of air (the partial oxygen pressure of five atmospheres of air approximates the total oxygen pressure of one atmosphere of oxygen) for the same time that they were confined in one atmosphere of oxygen. Five atmospheres of air, in every instance, gave exactly the same result as one atmosphere of oxygen. Thus, the toxicity of oxygen is unaffected by the presence of nitrogen and the rare gases of the air. In another experiment 3.5 atmospheres of air were used with the result that

the protozoa were not killed at all. It is evident, then, that mere mechanical pressure does not kill the protozoa.

2. *Cockroaches.*

Since the protozoa of termites were so easily removed by oxygenation, it immediately became desirable to try the method on other protozoa-harboring insects. The cockroach has many protozoa and can be obtained easily in quantity. By pressing on the abdomen with the fingers or some mechanical instrument and forcing out some of the intestinal contents it is not difficult to determine just what protozoa an individual harbors, and the procedure does not injure the insect. Cockroaches with two ciliates, *Nyctotherus* and *Balantidium*, and two flagellates, *Lophomonas* and *Polymastix*, were oxygenated at 3.5 atmospheres. About 200 insects were used in these experiments. The minimum time required to kill all individuals of each of the four protozoan genera is given in Table II. It is interesting to note that the flagellates were both killed in 40 minutes, the same time required to kill the flagellates of the large Pacific Coast termite (*Termopsis*), at this pressure, while the ciliates were not all killed until 3½ hours, more than five times the time required to kill the flagellates living under identical conditions. From this it would appear that oxygen is actually more toxic for flagellate protozoa.

TABLE II.

TIME REQUIRED AT 3.5 ATMOSPHERES OF OXYGEN TO KILL ALL INDIVIDUALS OF CERTAIN INTESTINAL PROTOZOA OF

FROGS				COCKROACHES		
No. of frogs used	Protozoa	Range in hours	Mean in hours	Protozoa	Hrs.	Min.
15....	*Hexamitus*.....	3–7	5	*Lophomonas*.....		40
10....	*Polymastix*.....	5–11	7	*Polymastix*.....		40
35....	*Trichomonas*.....	8–15	12	*Nyctotherus*.....	3	30
30....	*Opalina*.....	12–20	18	*Balantidium*.....	3	30
3.....	*Nyctotherus*.....	28	28			

Two other protozoa, an unidentified flagellate and *Endamœba blattæ*, were present in some of the cockroaches, but not in a sufficient number to make it feasible to work out the minimum time required to kill them. They were killed.

When cockroaches are confined in oxygen at 3.5 atmospheres, they are able to live approximately 90 hours, which is 26 times as long as their ciliate and 135 times as long as their flagellate protozoa live at this pressure. Here, again, as in termites, it is evident that the oxygenation which removes the protozoa of cockroaches certainly does little, if any, harm to the cockroaches themselves. They, unlike termites, live normally (indefinitely) after their protozoa have been removed. Oxygen, then, at 3.5 atmospheres is 135 as toxic for the flagellates and 26 times as toxic for the ciliates living in cockroaches as it is for the insects themselves.

3. *Earthworms.*

Many earthworms, harboring a fairly large number of ciliates of the genus *Hoplitophyra*, were oxygenated at 3.5 atmospheres, but the minimum time required to kill their protozoa was not determined. It is more than six and less than twenty hours.

4. *Frogs.*

After all protozoa had been removed from three invertebrates, it seemed highly desirable next to oxygenate a cold-blooded vertebrate harboring many protozoa. For this work the frog (*Rana pipiens*) was selected.

Most frogs harbor an abundant protozoan fauna; two ciliates, *Opalina* and *Nyctotherus*, and four flagellates, *Trichomonas* (*Tritrichomonas* in the opinion of some investigators), *Chilomastix*, *Hexamitus*, and *Polymastix*, are usually present in a fairly large number of hosts; in fact, all are sometimes present in the same host. Two hundred frogs were procured and just before being used in experiments each individual was examined in order to ascertain what protozoa were present. This examination was made by attaching a No. 7 hard rubber catheter, cut off at the insertion end to four inches in length, to a 5 cc. Luer syringe; by inserting the catheter into the rectum it was possible to draw out all or any amount of the rectal contents, which, when examined under the microscope, revealed immediately what protozoa each frog harbored. Of course, the number of protozoa present was also ascertained at the same time.

When frogs were oxygenated at 3.5 atmospheres, it was found that some of their intestinal protozoa were killed more quickly

than others, so it became necessary to determine the minimum time required to kill all individuals of each protozoan genus, and in working this out it was noticed that the protozoa of a certain genus would be killed more quickly in one host than in another; consequently, in most instances, a fairly large number of hosts was used. The number of hosts used, the range in time and the mean in time required to kill the protozoa, are given in Table II. It has been impossible to think of a plausible explanation of why the protozoa of one frog are affected more adversely by oxygen than those of another. This was noticed when several frogs were oxygenated in the same flask at the same time. The same phenomenon was met in the oxygenation of termites and cockroaches. Perhaps more work will throw light on it.

It is interesting to note that it takes 28 hours to kill the ciliate *Nyctotherus* in the frog and 3½ hours to kill *Nyctotherus* in the cockroach. The flagellate *Polymastix* is killed in 40 minutes in the cockroach while the species of this genus that lives in the frog is not killed until approximately 7 hours. It would be most interesting, indeed, to cultivate *Nyctotherus* and *Polymastix* from both hosts and then subject them to the same oxygen pressure. It is very probable, though not certain, in view of the oxygenation studies of the frog *Trichomonas* in vivo and in vitro, that oxygen is actually more toxic for *Polymastix* and *Nyctotherus* in the cockroach.

Some frogs live as long as 65 hours in 3.5 atmospheres of oxygen, more than twice as long as their ciliate and five to six times as long as their flagellate protozoa.

Twenty tadpoles,[1] 2 with *Nyctotherus*, 3 with *Trichomonas*, 3 with *Opalina*, 4 with *Hexamitus*, and 8 with *Euglenamorpha*, were oxygenated at 3.5 atmospheres with the result that their protozoa were killed in approximately the same time as those of frogs. *Euglenamorpha* is not present in adult frogs, and it was primarily for this reason that tadpoles were oxygenated. This flagellate is very similar morphologically to plant-like free-living protozoa of the genus *Euglena* which, as will be seen later, must be oxygenated sixty five hours at 3.5 atmospheres before being killed, while

[1] The method used to determine what protozoa each tadpole harbored was simple: Each individual was placed to itself in a small vesel; very soon a considerable quantity of fecal material was passed, which, when macerated and examined under the microscope, revealed the protozoa harbored.

Euglenamorpha is killed in approximately thirty hours. It is impossible to say whether this difference in oxygen toxicity is due to environment or metabolism. Here is an interesting situation, calling for careful investigation.

5. *Goldfish and Salamanders.*

After having removed all protozoa from an air breathing cold-blooded vertebrate, the frog, it seemed expedient to oxygenate a water breathing vertebrate harboring many protozoa. Goldfish and salamanders (*Necturus*) were used for this purpose. Twenty young goldfish, harboring large numbers of intestinal flagellates of the genus *Hexamitus*, and ten young salamanders, harboring large numbers of intestinal flagellates of the genera *Trichomonas* and *Prowazekella*, were oxygenated at 3.5 atmospheres. The same thing occurred here as in the oxygenation of frogs, namely, some hosts lost their protozoa sooner than others; *Hexamitus* in some goldfish was killed in 4 hours and in others not until 5 hours; *Trichomonas* and *Prowazekella* in some salamanders were killed in 9 to 10 hours and in others not until 11 to 12 hours. In adult hosts it would probably take slightly longer to kill all protozoa in all hosts. In the material used in this study all individuals of *Trichomonas* and *Prowazekella* were killed in all hosts in 12 hours and all individuals of *Hexamitus* in all hosts in 5 hours, while the hosts were not killed before 50 to 60 hours.

It is interesting to note how closely these death points of *Hexamitus* and *Trichomonas* inhabiting the water breathing vertebrates, salamanders and goldfish, parallel those of the *Hexamitus* and *Trichomonas* that inhabit the air breathing vertebrate, the frog (see Table II).

It is quite probable that the external parasitic ciliates of fish would be killed by oxygenation and without injury to the fish. These and other protozoan parasites, according to reports, do considerable damage to fish, which oxygenation would probably check.

What effect oxygenation would have on the hundreds of species of sporozoa in fishes should be determined.

6. *Rats.*

The next logical step in the development of the work was to oxygenate a warm-blooded vertebrate. *Trichomonas* (*Tritrichomonas* in the opinion of some investigators) is present in a fairly large number of rats; because of this, and owing to the ease with which rats may be obtained, the rat was selected. In order to demonstrate the presence of protozoa, fecal contents were removed by means of a catheter, as in the experiments with frogs, and were examined under the microscope.

It was found, however, that the rats themselves were not able to live more than five to six hours at 3.5 atmospheres of oxygen and that their protozoa were not killed in this time.

7. *Trichomonas from Frog, Rat and Man in Culture.*

Since it was impossible to remove the protozoa from a warm-blooded vertebrate by the method employed in removing them from the cold-blooded vertebrate, the problem of the toxicity of oxygen for the protozoa living in the intestines of rat and man was attacked in another manner, viz. the protozoa were grown in culture [1] and the cultures were oxygenated at 3.5 atmospheres by placing a few drops of the fluid from each culture in the same flasks that had been used in all the other experiments.

It was found (see Table I.) that the *Trichomonas* of frogs was killed in six hours, the *Trichomonas* of rats in ten hours, and the *Trichomonas* of man in eleven hours. Obviously, it is impossible, then, to kill the protozoa of the rat and of man by oxygenation at this pressure without killing the hosts themselves first.

It is interesting to note that the frog *Trichomonas* in culture is killed in about one-half the time required to kill it in the frog. This is perhaps explained in part by two facts: (1) oxygen is more soluble in water than in blood, and (2) the host furnishes some sort of resistance or barrier which makes it slightly more difficult for the oxygen to reach the protozoa.

[1] Several of the culture media that have been employed by other investigators were used, but the following medium, which is largely a compilation from other methods, was found to be very satisfactory. For frog *Trichomonas*, sodium citrate 1 per cent, sodium chloride 0.5 per cent, Löffler's dehydrated beef serum 0.5 gram, distilled water 100 cc.; for *Trichomonas* of man and rat, 0.2 per cent, more NaCl was used. Growth was very abundant. Subcultures were made every three days of the organisms from rat and man. The frog *Trichomonas* lived three months sometimes without being transferred.

These experiments, as well as those on free-living protozoa, show that oxygen is directly toxic for protozoa, that it acts on them directly and not through any tissues of their hosts. In other words, the tissues of the hosts are not stimulated by the oxygen to give off products which kill the protozoa.

8. *Free-living Protozoa.*

It seemed highly desirable to compare the toxicity of oxygen for free-living protozoa with that for parasitic or entozoic protozoa; consequently, several genera of flagellates and ciliates were selected and were oxygenated in the same manner as were the cultures of parasitic protozoa.

TABLE III.

APPROXIMATE MINIMUM TIME REQUIRED AT AN OXYGEN PRESSURE OF 3.5 ATMOSPHERES TO KILL ALL INDIVIDUALS OF CERTAIN FREE-LIVING PROTOZOA.

Ciliates	Hours	Flagellates	Hours
Paramoecium	5	*Euglena*	65
Chilodon	4	*Heteronema*	50
Diophrys	60		
Holostica	50		

The results of these experiments are given in Table III. The fact that *Paramœcium* is killed in 5 hours, *Chilodon* in 4, *Holostica* in 50, and *Diophrys* in 60 shows conclusively that oxygen is just as toxic for some free-living ciliates as it is for some parasitic ciliates and flagellates. It is actually even more toxic for *Paramœcium* and *Chilodon* than for *Trichomonas* of frog, rat, and man in culture; the former are killed in 5 and 4 hours respectively, while it required 6, 10 and 11 hours respectively to kill the latter. And we have seen that the frog *Trichomonas* in culture is killed in about half the time required to kill it in the frog, yet it is killed much sooner than the ciliates, *Opalina* and *Nyctotherus*. It has not been possible to cultivate *Nyctotherus* and *Opalina* from frogs, but, reasoning from the time required to kill *Trichomonas* in vivo and in vitro, it would take 10 to 12 hours to kill them in culture, or more than twice the time required to kill *Paramœcium* and *Chilodon*. I cannot explain why oxygen is less toxic for *Diophrys* and *Holostica* than for *Paramœcium* and *Chilodon*. Perhaps a combined study of the metabolism, habitat, and oxygen toxicity

of these and many other free-living ciliates will throw light on the problem.

After having oxygenated the ciliates, the results with the plant-like flagellates *Euglena* and *Heteronema* are not surprising. Some animal-like free-living flagellates would probably yield toxicity results quite similar to those obtained with parasitic flagellates.

Summary and Conclusions.

The toxicity of oxygen at various pressures for four genera of termites has been determined. At a pressure of 3.5 atmospheres the protozoa are all killed in two genera in 30 minutes, in one in 35 minutes, and in another in 40 minutes, while the termites themselves are not killed until 45 hours. Thus, oxygen is more than forty times as toxic for the protozoa as it is for the termites. This makes it possible to remove all protozoa from termites very easily and without injury to the host.

The protozoa of two termite genera were not killed at one atmosphere of oxygen even in ten days, while in two other genera they were killed in one and three days respectively. This gave an excellent opportunity to work out what effect, if any, partial pressures of other gases of the air, particularly nitrogen, had on oxygen toxicity. All four genera when confined in five atmospheres of air (partial O_2 pressure of 5 atms. of air approximates the total O_2 pressure of 1 atm. of O_2) gave exactly the same result as when confined in one atmosphere of oxygen for the same time. Thus, the toxicity of oxygen is in no way connected with or affected by the partial pressures of other gases of the air. It is the partial pressure of oxygen, and not mere mechanical pressure, that matters.

Cockroaches harbor many kinds of protozoa, all of which were removed by oxygenation at 3.5 atmospheres in $3\frac{1}{2}$ hours; the flagellates, *Lophomonas* and *Polymastix*, were killed in 40 minutes, and the ciliates, *Nyctotherus* and *Balantidium*, in $3\frac{1}{2}$ hours. The cockroaches themselves were not killed until 90 hours. Thus, oxygen at this pressure is 135 times as toxic for the flagellates and 26 times as toxic for the ciliates living in cockroaches as it is for the insects themselves.

It is highly probable that all insect-inhabiting protozoa may be removed by oxygenation without injury to their hosts. If so, the

rôle which insects play in the transmission of protozoa from man to man, from animal to animal, from animal to man and from plant to plant can be worked out much more effectively. What effect, if any, oxygenation would have on other insect-transmitted organisms, bodies, inclusions, and agents would be well worth study.

Earthworms when oxygenated lose their ciliates and are uninjured by the process.

Frogs harbor many protozoa. More than 150 experiments have been carried out on the oxygenation of frogs, and all the intestinal protozoa may be removed without injury to the frogs. Table II shows the minimum time required to kill three flagellates, *Hexamitus*, *Polymastix* and *Trichomonas*, and two ciliates, *Opalina* and *Nyctotherus*. The ciliates are killed in less than one-half the time required to kill the frogs, and the flagellates in one-fifth to one-tenth the time.

The protozoa of two water breathing vertebrates, goldfish and salamanders, were all killed by oxygenation in less than one fifth the time required to kill their hosts.

If oxygenation will remove the protozoa of other amphibia, it will be possible to make some interesting studies on protozoal host specificity.

It is highly probable that all intestinal flagellates and ciliates may be removed from all invertebrates and from all cold-blooded vertebrates by oxygenation and that none of these hosts will be injured. It is also possible that the sporozoa, amœbæ, and blood-inhabiting protozoa may be removed from the same hosts in the same way and without injury to the hosts.

Many experiments have been carried out on *Trichomonas* from frog, rat and man in culture. All of these protozoa are killed by oxygenation (see table I for the minimum time), but the time required to kill them in all except the frog is longer than it takes to kill the host itself at the same pressure; so it is impossible to remove the protozoa from rats and human beings by confining them in oxygen at 3.5 atmospheres. Perhaps oxygen may be successfully administered to warm-blooded vertebrates in some other way. Work of this nature is in progress.

Oxygenation experiments have been carried out on four genera of free-living ciliates and two of free-living flagellates. Oxygen

is certainly just as toxic for some free-living ciliates as it is for parasitic ciliates; for others, it is not. For *Paramœcium* and *Chilodon*, it is really more toxic; for *Diophrys* and *Holostica*, it is considerably less toxic. It is not very toxic for two plant-like flagellates, *Euglena* and *Heteronema*, but would probably be found to be just as toxic for some animal-like free-living flagellates as for some parasitic species.

Oxygen in excessive amounts is toxic for all animals, but protozoa possibly take up a correspondingly larger amount of it as the tension or pressure is increased than do higher animals and for this reason are affected more adversely than termites, cockroaches, earthworms and frogs. During oxygenation the protoplasm of the protozoa sometimes becomes very much vacuolated,[1] which may indicate that it is being consumed, perhaps actually burned up, by increased metabolism. However, the metabolism of higher vertebrates is said to be slowed down by increased oxygen pressure. But Amberson, Mayerson, and Scott ('24) were "able to show that the metabolic rate in some of the higher marine invertebrates, with well developed respiratory mechanisms, is closely dependent upon the oxygen tension in the water over a wide range."

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[1] It is also true that many dying protozoa, regardless of the cause of death, sometimes become vacuolated.